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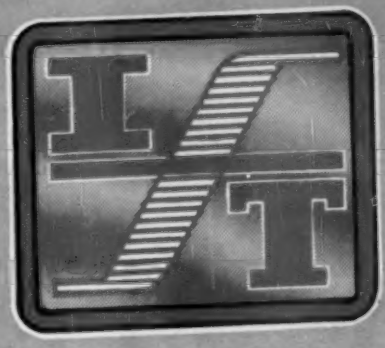

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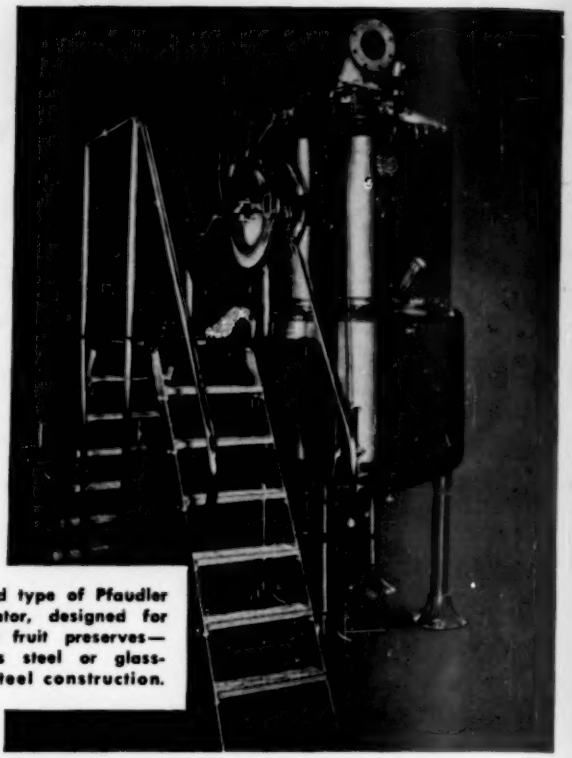

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Food Technology Curricula

This is the sixth of a series of articles which are intended to elucidate the basic ideas that govern the curricula of our educational institutions in the field of food technology.

Teaching of Sanitation in Connection With Food Technology Curricula[a]

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(Received for publication, May 29, 1950)

Food technology is a relatively new and expanding field of study. Twenty years ago only four or five universities offered instruction in this field.[1] In 1947, according to a survey made by Stewart, 16 colleges and universities were offering degree work in Food Technology. Since then the number has increased to nearer 30. Expansion in this field of instruction has been rapid and over a vast area of subject material. The curricula and programs of instruction are not standardized as in chemistry, botany and bacteriology.

In formulating the program for the Symposium on "Sanitation in the Food Industry," it was considered desirable to devote some time to a discussion of the teaching of sanitation in connection with Food Technology Curricula. Information on this subject was not readily available, so it had to be obtained by survey. Letters were sent to 43 institutions requesting information pertaining to the consideration of sanitation in connection with Food Technology Curricula. Each letter combined the following five questions:

1. Are definite courses in sanitation given under Food Technology?
2. Is sanitation taught in connection with courses in Food Technology?
3. Is it given in connection with other departments, i.e. Public Health?
4. Is it ignored?
5. Are students required to take sanitation courses? If so, how many semester hours, or if courses are suggested how many semester hours?

Replies were received from thirty-seven of the forty-three institutions. Of the six that failed to reply four are located in the South, and at least two (possibly 3) of these are concerned with some type of instruction in Food Technology.

The survey showed that the administration of Food Technology Curricula varies greatly with the institution. In several universities the teaching is conducted under well organized Departments of Food Technology. In others, it is given under the auspices of other interested departments, such as Horticulture, Chemistry, Biochemistry, Bacteriology, Dairy Industry, etc. In still other cases the teaching of Food Technology cuts across several departments and is administered by a committee representing these departments. In such cases there may or may not be formal courses in Food Technology, or instruction may be limited to the Graduate level. In cases where formal courses in Food Technology are not given, courses in related fields such as Chemistry, Biochemistry, Bacteriology, Engineering, Biology, Public Health, or even Veterinary Science are slanted toward food technology. These factors pertaining to organization are of interest here since they undoubtedly influence the extent and procedures of teaching sanitation in connection with Food Science.

Answers to the specific questions circulated are summarized in Table 1. It should be pointed out, however, that answers were not always clear, and in a number of cases it was necessary to use judgment in interpretation.

TABLE 1

Present Status of the Teaching of Sanitation in Thirty-seven Institutions Teaching Food Technology

Question	Answers	
	Yes	No
1. Are courses in Sanitation given under Food Technology?	1 [b]	29
2. Is Sanitation taught in connection with Food Technology courses?	18	16
3. Is it given in connection with other Departments?	29	4
4. Is it ignored?	....	24
5. Are students required to take courses in Sanitation?	8 [c]	24

[b] Two departments plan to include courses in the near future.
[c] Two will require courses in the future.

It is apparent that all schools give some consideration to sanitation in their respective Curricula in Food Technology. Formal courses in Sanitation are offered under the auspices of Food Technology Departments in seven universities. In other cases the subject is covered by devoting a limited amount of time in Food Technology courses to the subject, or in departments representing closely related fields such as Chemistry, Biochemistry, Bacteriology, Engineering, Public Health and Veterinary Science.

The amount of time devoted to teaching sanitation varies greatly with the institution. In cases where formal courses are devoted to the subject, it ranges from three to four hours (with or without laboratory) per week for one or two semesters. It is hard to say just how much time is devoted to sanitation when it is covered in Food Technology courses. Under such circumstances, however, the time and effort is very much less than when formal courses are given in the subject.

The subject matter covered appears to vary with the emphasis and interest of each particular Department of

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

Food Technology, or whether or not it is covered in related courses. There seems to be an emphasis on rodent control, insects, detergents, and possibly water pollution, when covered in Food Technology courses. When the teaching is given in courses in other departments, however, there is more emphasis on water, sewage, and waste disposal. It is uncertain as to whether or not ventilation, heating, lighting and housing receive much attention. Apparently the consideration of food borne infections could be more generally emphasized.

In conclusion it may be said that the general field of sanitation is considered important and receives attention in the teaching of Food Technology. The time and effort devoted to his field, however, varies greatly with the particular university. There appears to be a divergence of opinion with respect to the subject matter that should be covered in the discussion of sanitation. For example, in one case the point of view is to cover the subject very thoroughly in two inclusive courses. In another no formal courses are offered or required and the point of view is indicated by the following statement: "I classify knowledge as the ability to reason, think clearly, apply common sense and apply fundamental principles of science rather than information about science."

It appears that there is a need for a better definition of what sanitation should include and what should be emphasized. Perhaps this is a place where the Sanitarians can help those engaged in teaching Food Technology.

COMMENTS BY B. E. PROCTOR

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The survey that Dr. Mrak has undertaken would be a commendable project if it accomplished nothing more than to demonstrate that a widely varying degree of emphasis is placed on the importance of including instruction in the principles of sanitation as a part of formal training in food technology.

In my opinion, this survey has accomplished more than this. It has also very definitely indicated (in Table 1) that an overwhelming majority of those responsible for the planning and the administration of food technology courses have already made provision in some way for instruction in sanitation to be included in the food technology curriculum. This signifies that the essential importance of instructing embryonic food technologists in matters of sanitation is recognized almost universally.

The need for a proper concept of the importance of sanitation in food manufacturing processes is increasing each year. Many a food industry has suffered serious reverses in the past because of lack of understanding of sanitation principles and lack of instruction in sanitation on the part of its key technical personnel. If correct emphasis were placed on instruction in sanitation in the education of future food technologists, food industries would begin to benefit from such instruction and the efficiency of those industries that are now doing a good job from the standpoint of sanitation would be increased.

What constitutes the proper amount of this type of instruction for students of food technology, what may be the optimum limits of such instruction, and what specific agency should be responsible for this phase of instruction are variables that are bound to differ from institution to institution and even from individual to individual in a single instituton.

Equally controversial is the question whether sanitation subjects are best taught by specialists in sanitary science, bacteriology, or public health, or by instructors in food technology courses into which problems in sanitation have been woven, although not listed as such.

Perhaps at this stage we should be less concerned with how much instruction in sanitation is necessary and how or by whom the instruction is given than with how effectively the end objective of such instruction is realized, namely, of making the student sanitation-conscious and effective when his studies are completed and he goes out into the world of food production.

The chances are that no institution does more than it should in this phase of its training of food technologists and that some institutions may be doing much less than is desirable. Yet every serious teacher of future food technologists who reads this will think, and perhaps ask, "How can we do any more and yet do what we need to in all the other phases of the food technology curriculum, with only a 4-year period in which to do it?"

The problems relating to instruction in sanitation for food technologists are not unlike the problems brought to light in the Stewart survey in respect to other subject matter in food technology, where wide variation in subject matter was shown to exist in various institutions. It is also necessary, therefore, to think more specifically about the relative importance of other subjects now found in food technology curricula.

Complete uniformity in the training of food technologists in all schools and universities offering food technology curricula would be impossible and very likely undesirable, even if it were possible.

If certain minimum levels of accomplishment could eventually be agreed upon for instruction in sanitation, —and other instruction in food technology,—this would probably in the long run be a boon to students and to those educators who are most earnestly striving to do a better job of training such students.

The establishment of minimum levels in sanitation knowledge as well as technical knowledge in other areas should, last but not least, benefit the food industries who have a right to assume that the food technologists they hire and on whom they depend for future progress have attained an adequate background and training, which in turn represents an asset of definite and established value.

The time may not be too far away when the professional food technologists may take a leaf from the book

of the Institute of Chemical Engineers, a professional society only a few decades older than the Institute of Food Technologists. The Institute of Chemical Engineers finally decided that the best method of guidance and control of the training of their future members was to set up a system of evaluating and accrediting the institutions of learning that present courses in chemical engineering. In their case this has worked out satisfactorily, although a period of adjustment was necessary to determine properly, by common accord, how a chemical engineer should be trained.

Surveys of this type are most helpful aids, on occasion serving the purpose of indicating possible errors of omission or commission by the curriculum organizers and the teaching fraternity. In addition, in the case of a profession of as recent origin as food technology, which is still in a state of flux, such surveys should serve as an inspiration for dynamic thinking and as a stimulus to strive for a more perfect goal, based in part on the experience of others. The patient efforts of those who originate and conduct such surveys should be most gratefully appreciated.

A Study of the Factors Affecting Measurement of Headspace in Canned Corn

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(Received for publication, May 29, 1950)

Using canned corn as an example, the factors influencing the accuracy and reproducibility of headspace measurements were investigated.

INTRODUCTION

The measurement of headspace is in common use for defining the fill of container in canned foods. Ordinarily the headspace is determined after opening the can with a can opener which leaves the double seam intact. Measurements are then made from the top of the double seam vertically downward either to the level of liquid or to the level of product in the can.

A machinist's depth gauge may be used alone to measure headspace or it may be used in conjunction with a circular perforated plate which rests on the surface of the product and provides a firm surface to which the measurement is made from the top of the can. A correction is added to account for the thickness of the perforated plate. More elaborate devices are usually designed to assure measurements parallel with the can axis.

Dodge *(2)* and others in the Bureau of Chemistry, United States Department of Agriculture, about 1917 assembled a device now known as the "Dodgometer." This instrument consists of a graduated rod one end of which is fastened at right angles to the center of a perforated disk which varies with can size. In using the Dodgometer, the perforated disk is placed on the leveled surface of the solid material in the can. Then a bar is placed across the top of the can, and the headspace is determined as height from the surface of the product to the top of the can. A variation of this instrument was constructed in which the graduated rod was passed through a snug fitting bearing at the center of a large leveling plate. Upon placing this plate on the top of the open can, the rod and its attached perforated disk were respectively aligned at right angles and parallel with the top of the can. Recently Smith *(4)* has modified the Dodgometer by addition of a weight (2.5 pounds for a No. 2 can) to the instrument in an effort to define compactness of fill.

The headspace as a factor in processing canned foods has been considered by at least two investigators. Yourga *(6)*, working with glass containers, determined a relationship between headspace volume, expressed as percent of container weight capacity, and headspace depth, measured vertically from the top of the container. Expressed in graphical form, this information permits rapid conversion of conventional headspace values to percent of container capacity. This conversion is of importance in estimating the maximum internal pressure in glass containers during processing. Merrill *(3)* presented an alignment diagram relating initial vacuum, sealing temperature, temperature rise within the container during processing, headspace measured as percent of overflow capacity of the container, processing temperature and maximum internal pressure developed in the glass container during processing.

The purpose of the present study was to investigate the sources and magnitudes of the errors associated with the measurement of headspace. Practically no evidence concerning the accuracy and reliability of headspace measurements has been presented in the literature. In view of the importance of this factor in food processing, it seemed desirable to define some of the errors inherent in headspace measurement.

EXPERIMENTAL PROCEDURE AND RESULTS

The reliability of headspace measurements depends on many factors which may be considered in three general groups: (1) commodity factors, (2) processing factors and (3) measurement factors.

The commodity factors have been reduced to a minimum in this investigation through consideration of one commodity: canned sweet corn, packed as cream style, whole kernel brine pack and as vacuum pack. The methods employed should be of use in similar investigations on other commodities.

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[a] Journal Paper No. J-1789 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 1052.

[b] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

The processing factors investigated were the influence of (a) size of headspace on the accuracy of headspace measurement, (b) style of pack and (c) can size.

The measurement factors included (a) comparison of three types of measuring instruments and (b) influence of the operator who levels the surface to which the measurement is made.

In all cases the headspaces were measured in 32nds of an inch. The depth gauge and plate were used for measuring headspace except where instrument comparisons were made. All results were subjected to analyses of variance (5) as a means of evaluating the importance of several sources of variability upon the accuracy of headspace measurements.

MAGNITUDE OF HEADSPACE

Two series of ten cans each were leveled by one operator. The headspace was measured by each of six operators, each operator making three independent measurements on each can. The original headspaces were retained in the first series. In the second a range from 16/32 to 44/32 was introduced by removing varying amounts of corn and liquid.

In general, the data indicated no important differences attributable to the range of headspace investigated. The individual operator mean squares presented in Table 1 indicate that five out of six operators made readings with only small variability while the sixth operator (No. 2) made readings which varied by relatively large amounts.

Comparisons of the Can × Operator (C × O) and Readings (R) mean squares indicated that an operator estimates the headspace of a single can with approximately the same accuracy as different operators estimate the headspace of a single can.

The concept of variance components *(1)* may be introduced to permit the examination of different situations respecting the variance of a mean. As an example consider the effect of varying sample size as well as taking one or more readings per sample, the appropriate formula is:

$$\frac{1}{n}\left(\frac{s_R^2 + s_{OC}^2 + s_C^2}{r}\right)$$

where r is the number of readings per can and n the number of cans in the sample. Using estimates of the components obtained from the data in Table 1, the following variances were constructed:

n = number of cans	r = number of readings		
	1	2	3
10	2.08	2.03	2.01
50	0.42	0.40	0.40
100	0.21	0.20	0.20

It should be noted that, in order to affect large reductions in the variance of the mean, the number of cans must be increased. By comparison, increasing the number of readings has only a negligible effect.

PREPARED AND MARKET SAMPLES

The difficulties encountered in obtaining market samples of cream style, whole kernel brine pack and vacuum pack corn in the four can sizes considered, emphasized the need for information concerning the comparative reliability of market samples and samples prepared in the laboratory. In an effort to clarify this point, market samples of the three styles of corn in No. 2 cans were compared with samples in No. 2 cans which were prepared in the laboratory. The data indicated no marked changes in variability between prepared and market samples.

TABLE 1

Analyses of Variance of Normal and Altered Headspace Measurements of Commercial Canned Corn Samples

Source of Variation	Degrees of Freedom	Mean Squares for	
		Normal Headspace	Altered Headspace
Operators (O)	5	29.50	23.00
C × O	45	1.61	3.51
Readings	120	1.05	1.04
Operator 1	20	0.40	0.57
Operator 2	20	2.45	4.13
Operator 3	20	1.10	0.87
Operator 4	20	1.20	0.27
Operator 5	20	0.63	1.60
Operator 6	20	0.50	0.23

CAN SIZE AND STYLE OF PACK

In the investigation of processing factors, four samples of each style of pack (whole kernel brine, vacuum pack and cream style) were prepared in the laboratory in No. 1, No. 303, No. 2 and No. 10 size cans. From examination of the data obtained, no clearly defined influence of either size of can or style of pack could be detected.

LEVELING ERROR

Prior to the measurement of headspace, the operator levels the surface of the commodity and may introduce variation as a result of either leveling or measuring. A factorial experiment was designed to determine the error introduced by leveling and by measurement as two steps necessary in determining headspace. Leveling refers only to leveling the surface of the product, not to the leveling of the can. In addition, a third factor (the influence of two different measuring devices) was introduced. Four cans of whole kernel brine pack corn were used. Each of four operators was required to level the surface of the product in each can, then each of the operators, in a pre-assigned order, measured the headspace of each can using two instruments, namely, (1) the perforated plate and depth gauge and (2) the Dodgometer equipped with leveling plate. This procedure was repeated four times so that each of the operators leveled the surfaces of each can and all four measured after each leveling. The contents of the cans were disturbed in a more or less uniform manner before each leveling.

The analysis of variance (Table 2) indicates that the operation of leveling accounts for most of the operator variation. Conversely, while the Measurement (M) and the Leveling × Measurement (L × M) interaction mean squares show significance, they account for only a small part of the operator variation.

The two instruments gave a rather consistent difference in headspace readings, with ten out of the 16 operator combinations giving higher readings for the Dodgometer. The differences were not excessive and could easily result from differences in the weights of the two instruments.

TABLE 2

Analysis of Variance of Influence on Headspace Measurements of the Operator Leveling the Commodity Prior to Measurement

Source of Variation	Degrees of Freedom	Mean Squares
Operator Combination (OC)	15	
Leveling (L)	3	54.28
Measuring (M)	3	3.15
L × M	9	3.89
Error (a)	45	0.72
Instruments (I)	1[1]	4.13
I × OC	15	0.63
Error (b)	48	0.52

COMPARISON OF THREE MEASURING INSTRUMENTS

In the measurement of headspace of products where the degree of compactness of fill is in question, the weighted Dodgometer proposed by Smith *(4)* has a logical application. The two instruments used previously are not designed to measure compactness of fill. To obtain some estimate of error for the weighted Dodgometer, four cans were measured by two operators making triplicate readings on each can with each of the three instruments. In the use of the weighted Dodgometer an increase in the time of settling allows an increase in the observed headspace. As a result all readings were made after the weighted Dodgometer had been permitted to settle for 30 seconds. Readings made with this instrument were approximately 16/32 of an inch greater than those resulting from the use of either of the other instruments.

The Readings standard deviations for the three instruments were 0.79, 1.38 and 1.12 32nds of an inch for the perforated plate and depth gauge, Dodgometer with leveling plate, and weighted Dodgometer, respectively. In terms of coefficients of variation the values are 3.3, 5.6 and 2.7 percent for the above standard deviations. The unweighted Dodgometer, the only instrument of the three used which is provided with a device for maintaining the measuring bar on the can axis, displayed the greatest variability. Considered in conjunction with the leveling factor, this fact suggests that, since the leveling operation introduces a fairly large error, an instrument not possessing a positioning device gives greater accuracy than the Dodgometer. This is probably true only when the measurements to the disk are made normal to the midpoint of the axis of slope of the perforated plate.

DISCUSSION

The purpose of this investigation was to determine the magnitude of the errors associated with determination of headspace in canned corn. Two types of variation have a bearing on the accuracy of measurement: (1) the ability of an operator to obtain close agreement among independent readings of the same headspace and (2) the consistency of the deviations after accounting for operator and can differences. Reliable estimates of both of these were obtained. In terms of standard deviations, the upper limit may be taken as about 2/32 inch for either one. This indicates that an operator could be expected to estimate the headspace of a single can of corn within 2/32 of an inch.

The chief source of variability in the determination of headspace is the operator. Given cans to level, it was found that differences in headspace due to leveling ranged up to 2.8/32 for a group of four operators. Similarly, given cans to measure after having been leveled by someone else, headspace measurements differing as much as 2.7/32 were encountered among a group of six operators. Thus, if the errors described are assumed to be additive, differences up to 5 or 6 32nd of an inch may reasonably be expected in measurements of the same can by two different operators due to their differences in leveling and measuring characteristics. All of this is in addition to the 2/32 of an inch standard deviation cited above.

In making routine determinations of headspace, standardization of procedure should minimize the effects of these different sources of variation.

SUMMARY

The factors responsible for variations in headspace measurements may be divided into three groups: (1) commodity, (2) processing and (3) measurement factors.

Variations in magnitude of the headspace had no influence on the accuracy of its determination.

The substitution of samples prepared in the laboratory with indefinite increments of headspace for commercial (market) samples added latitude to the investigation and accrued no additional variations.

Differences between operators were rather consistently encountered.

The operators leveling the surface of the product were able to influence markedly the magnitude of headspace measurements.

The standard error of a single observation, i.e., the standard deviation, should be taken as 2/32 inch to accommodate most operators. Using this value, any headspace falling within 2/32 inch of a headspace limit (e.g. allowable slack-fill limit) would be open to question. If the variation due to leveling and to operators is included, this limit must be raised to 6-8/32 inch.

A comparison of the depth gauge and plate method of measurement with the Dodgometer and the weighted Dodgometer indicates that variations of approximately three percent result from use of the depth gauge and plate and from the weighted Dodgometer, while use of the un-weighted Dodgometer may allow variations of approximately six percent.

The three instruments tested all indicate associated standard errors not in excess of the 2/32 inch value suggested as an upper limit.

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Studies on In-Plant Chlorination[a]

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Investigations were made at four canneries to study the effect of in-plant chlorination on bacterial populations and slime formation at various places in the canning lines. Free residual chlorine levels between 2 and 10 p.p.m. were applied. The products studied were peas, corn, cherries, apricots, peaches, pears and fruit cocktail. Lower populations of mesophilic aerobic bacteria occurred in equipment when in-plant chlorination was used than when it was not used. In-plant chlorination also prevented the formation of slime on equipment, on floors, in gutters, around closing machines, etc., and eliminated plant odors.

Chlorine used at residuals of 4-5 p.p.m. had no detectable effect on flavor of 26 canned fruits and vegetables, provided the water for fruit sirups was unchlorinated.

Apples, figs, peaches (clingstone), pears, strawberries, and yams were most sensitive to chlorine from a flavor standpoint. No increased corrosion was attributed to chlorination where a residual of 5 p.p.m. was maintained during operations, and 20 p.p.m. for clean-up.

INTRODUCTION

Chlorinating the water supply of a food manufacturing plant beyond the "break-point" is known as "in-plant chlorination." Griffin *(4)*, has explained break-point chlorination as the addition of sufficient chlorine to completely satisfy the demand of the water, and permit free residuals to be produced and maintained in the distribution system.

Hall and Blundell *(5)* indicated the potentialities of in-plant chlorination in a cannery as an aid for preventing slime formation and other microbial growth. Their conclusions were based on observations of conditions in the canning of peas and corn and on preliminary results of bacteriological studies made by the National Canners Association Western Branch Research Laboratories. The bacteriological data were not published at that time pending confirmation of the results by tests on other products. Similar beneficial effects have been reported by Brownlee *(3)*, Ritchell *(8)*, Hurley *(6)*, and others *(1)*.

The introduction of in-plant chlorination, and the indication that it might have extensive applications in food plants as a sanitary control procedure, raised the following questions: (1) Are the benefits of its use sufficient to justify the cost; (2) Will chlorine, in the concentrations recommended, have any adverse effect on the quality and flavor of canned foods; and (3) Will corrosion of the equipment be accelerated? The work reported in this paper was undertaken to investigate these questions by field studies, experimental packs, and observations of the effects of in-plant chlorination in canneries over a period of five years.

FIELD STUDIES

Plan of Experiments. Field studies were made in a pea cannery, a corn cannery, and two fruit canneries packing clingstone peaches, pears, fruit cocktail, apricots and Royal Anne cherries. The observations cover three consecutive phases of chlorine treatment as follows: (1) three or four days with chlorination, (2) three or four days without chlorination, and (3) three or four days with the chlorine on again. With pears, clingstone peaches and fruit cocktail, the first phase was eliminated to fit the cycle into a week's operation, because the plant was shut down every Sunday for a thorough clean-up. It was considered that any beneficial effects shown by such a series would be conclusive, since the plant was operated for three days following the clean-up before the chlorine was turned on.

All field studies were made with the plants operating at their normal capacities, using fresh water for all purposes except in pea washers where the water was reused. However, considerable fresh water was continually added to these washers. The clean-up consisted only of a low pressure cold water wash, in order that the results would not be too greatly affected by it. The product conveyor belts and bucket elevators in each plant were continually sprayed with water on the return side. Chlorine was used in gaseous form and was dispersed through an automatic proportionating chlorinator at levels shown in the figures and tables together with the results.

The studies included total plate counts of mesophilic aerobic bacteria, slime surveys, and general observations on cleanliness and odors.

Bacteriological Procedure. Plate counts were made on glucose-tryptone-agar (1 percent tryptone, 0.5 percent glucose, 1.5 percent agar, with bromo-cresol-purple indicator), with incubation at 30° C. for 48 hours. The results were expressed as bacteria per ml. of water or product, assuming a density of 1.0 for cream style corn. Any chlorine in the sample was neutralized by adding 0.5 ml. of sterile 1.5 percent aqueous solution of sodium thiosulfate to each 6 oz. sample bottle.

Except for cream style corn, the water in which the product was held or conveyed was taken as the sample rather than the product itself. This gave an indication of the contamination around the product. It was felt that such results would be less variable than if the product itself were sampled on a weight basis, since there is usually considerable variation in product particle size which would alter the relationship of weight to surface area exposed.

About 150 ml. was collected in a sterile 6 oz. bottle for each liquid sample. Closed cans were thoroughly shaken before sampling, opened aseptically, and the brine or sirup poured into sterile bottles. For cream style corn, 50 g. of each sample was weighed aseptically into a bottle containing 200 ml. of sterile water and the bottle thoroughly shaken. Control plates were poured twice each day as a check on contamination.

For peas, corn, peaches, pears and fruit cocktail, two sets of samples were taken each day: one about two hours after operations started, and the other about two hours after the noon shut-down. The results of these two samplings were averaged to give the daily counts.

With apricots, it was possible to obtain samples only on one day, with chlorination in the morning and without it in the

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

afternoon. The tests on Royal Anne cherries were also made in one day and included a complete cycle, but the reverse of that used for peas and corn (see Figure 3).

The sampling points for the various products are indicated with the results. The samples were taken in a sequence so that approximately the same product was passing through successive pieces of equipment at the time of sampling.

RESULTS AND DISCUSSION OF BACTERIOLOGICAL FIELD STUDIES

The effects of chlorination on bacteriological counts in pea, corn, and cherry canning are demonstrated graphically in Figures 1, 2 and 3, and the data for apricots, peaches, pears, and fruit cocktail are given in Tables 1, 2 and 3.

The points on the graphs for peas and corn were obtained by averaging the counts for each day and then averaging all the daily counts for each phase of chlorine treatment.

The plate counts for peas and corn were much lower when chlorine was used than when it was not used. The most pronounced reductions in counts occurred in equipment such as washers, flumes, and graders, where fairly large volumes of fresh water were continually added. At the end of the period without chlorination in the pea cannery, the bacterial populations had built up to high levels in the filled cans and in much of the equipment.

The daily washing of the pea blancher with chlorinated water kept it clean and low counts were obtained. These increased a hundred-fold, however, when the

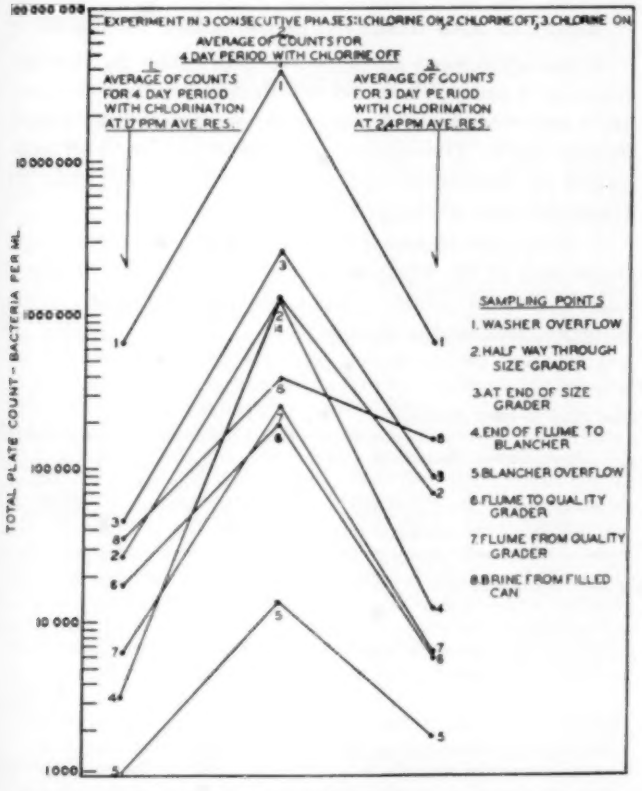
EXPERIMENT IN 3 CONSECUTIVE PHASES: 1 CHLORINE ON, 2 CHLORINE OFF, 3 CHLORINE ON
AVERAGE OF COUNTS FOR 4 DAY PERIOD WITH CHLORINE OFF
AVERAGE OF COUNTS FOR 4 DAY PERIOD WITH CHLORINATION AT 1.7 PPM AVE. RES.
AVERAGE OF COUNTS FOR 3 DAY PERIOD WITH CHLORINATION AT 2.4 PPM AVE. RES.
TOTAL PLATE COUNT - BACTERIA PER ML.
SAMPLING POINTS
1. WASHER OVERFLOW
2. HALF WAY THROUGH SIZE GRADER
3. AT END OF SIZE GRADER
4. END OF FLUME TO BLANCHER
5. BLANCHER OVERFLOW
6. FLUME TO QUALITY GRADER
7. FLUME FROM QUALITY GRADER
8. BRINE FROM FILLED CAN

FIG. 1. Effect of in-plant chlorination on total counts in a pea cannery. Sampling points 2 and 3 were included because the peas were flumed or bucket-conveyed from one grader screen to the next. The peas passed through an elevator, over a table, and through a filler between samples 7 and 8.

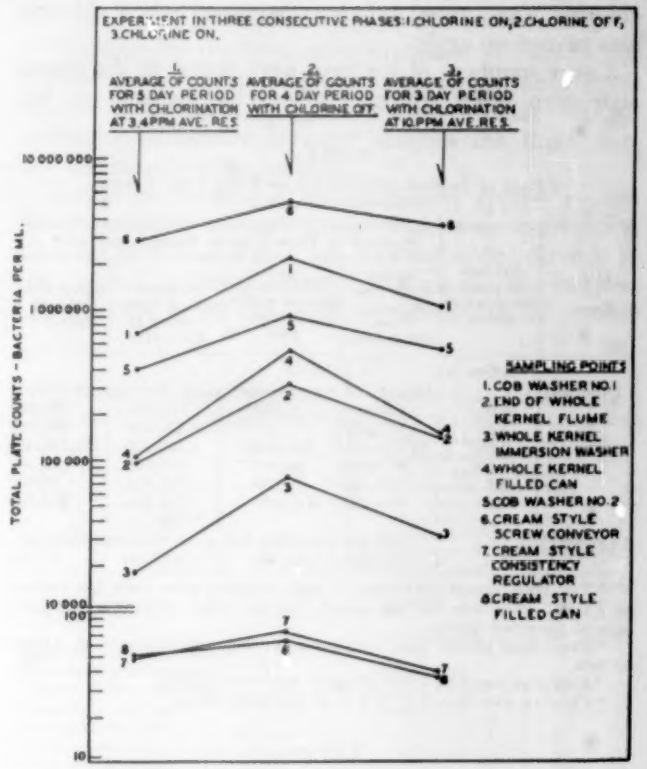
EXPERIMENT IN THREE CONSECUTIVE PHASES: 1 CHLORINE ON, 2 CHLORINE OFF, 3 CHLORINE ON.
AVERAGE OF COUNTS FOR 5 DAY PERIOD WITH CHLORINATION AT 3.4 PPM AVE. RES.
AVERAGE OF COUNTS FOR 4 DAY PERIOD WITH CHLORINE OFF.
AVERAGE OF COUNTS FOR 3 DAY PERIOD WITH CHLORINATION AT 10 PPM AVE. RES.
TOTAL PLATE COUNTS - BACTERIA PER ML.
SAMPLING POINTS
1. COB WASHER NO. 1
2. END OF WHOLE KERNEL FLUME
3. WHOLE KERNEL IMMERSION WASHER
4. WHOLE KERNEL FILLED CAN
5. COB WASHER NO. 2
6. CREAM STYLE SCREW CONVEYOR
7. CREAM STYLE CONSISTENCY REGULATOR
8. CREAM STYLE FILLED CAN

FIG. 2. Effect of in-plant chlorination on total plate counts in a corn cannery. Cob washer No. 1 was for whole kernel line, and cob washer No. 2 was for cream style line. The corn passed over a picking table, through an elevator, and through a filler between samples 3 and 4.

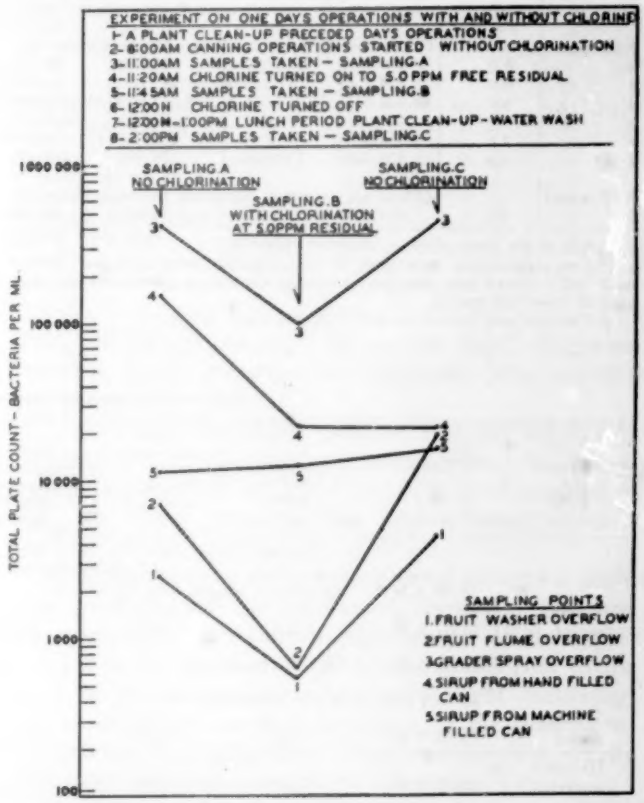
EXPERIMENT ON ONE DAYS OPERATIONS WITH AND WITHOUT CHLORINE
1- A PLANT CLEAN-UP PRECEDED DAYS OPERATIONS
2- 8:00AM CANNING OPERATIONS STARTED WITHOUT CHLORINATION
3- 11:00AM SAMPLES TAKEN - SAMPLING-A
4- 11:20AM CHLORINE TURNED ON TO 5.0 PPM FREE RESIDUAL
5- 11:45AM SAMPLES TAKEN - SAMPLING-B
6- 12:00N CHLORINE TURNED OFF
7- 12:00N-1:00PM LUNCH PERIOD PLANT CLEAN-UP-WATER WASH
8- 2:00PM SAMPLES TAKEN - SAMPLING-C
SAMPLING A NO CHLORINATION
SAMPLING B WITH CHLORINATION AT 5.0 PPM RESIDUAL
SAMPLING C NO CHLORINATION
TOTAL PLATE COUNT - BACTERIA PER ML.
SAMPLING POINTS
1. FRUIT WASHER OVERFLOW
2. FRUIT FLUME OVERFLOW
3. GRADER SPRAY OVERFLOW
4. SIRUP FROM HAND FILLED CAN
5. SIRUP FROM MACHINE FILLED CAN

FIG. 3. Effect of in-plant chlorination on total plate counts in a cherry cannery. Sample No. 3 was taken because water was used as a lubricant on the grader.

chlorine was turned off, but decreased when chlorine was turned on again.

Large numbers of bacteria were found in the cream style corn screw conveyor when chlorine was on, but the counts were even higher when the chlorine was off. This difference was attributed to the use of chlorinated water for the daily clean-up.

In general, the bacterial populations were much lower in the fruit canneries when chlorine was on than when it was not used. The counts from most sampling points for peaches, pears and fruit cocktail were extremely high on the third day of operation without chlorination, but were considerably reduced after one day's operation with it.

The rapid effects of chlorine on bacterial populations in fruit canning equipment were clearly demonstrated by the results obtained on a single day's operations in a cherry cannery (Figure 3). When the cleaned plant was allowed to run three hours in the morning without chlorination, high counts were obtained, but 25 minutes after the chlorine was turned on the bacterial population showed a marked decrease. When the chlorine was turned off again in the afternoon, high counts resulted.

Counts on sirup from closed cans of fruit were variable and did not show any consistent relationship to chlorination. This may have been due to the cans being held on belts for varying lengths of time before siruping, or to the fruit being held in sinks prior to canning.

The value of in-plant chlorination in canneries for keeping the numbers of aerobic mesophilic bacteria at a low level was clearly demonstrated by these tests. A chlorine residual of about 5 p.p.m. in the plant water supply seemed to be adequate for the prevention of slime and the control of bacterial growth in the water and equipment.

TABLE 1

Effects of In-plant Chlorination on Total Plate Counts: Clingstone Peaches

Date of Expt.	Average in-plant chlorination levels	Average Plate Counts, Bacteria per ml.[b]			
		Shaker spray run-off, water from pitted fruit	Drum spray run-off water, after lye peel	Grader run-off water (spray to lubricate)	Sirup from canned halves [c]
	p.p.m.				
1 (Sunday)	....	(Plant not operating but given thorough clean-up)			
2	0.0	3,600,000	1,220	133,000	100,000
3	0.0	1,517,000	..... [d]	9,320,000	181,000
4	0.0	22,130,000	348,800	1,260,000	131,258,500
5	4.5 [e]	914,000	40,500	634,000	198,000
6	3.5	399,000	25,000	891,000	86,000
7	4.0	924,000	147,000	748,000	226,000
8 (Sunday)	....	(Plant not operating but given thorough clean-up)			
9	5.0	662,000	105,000	440,000	53,000

[b] Two plate counts were made at each sampling point each day (except on 2 and 7 when only one was made), and the values given here are averages of these two counts.

[c] Fruit hand-packed from canning sinks and cans conveyed to siruper on belt.

[d] Excessive count at 1:100 dilution, was not counted.

[e] Chlorine was turned on at 9 P.M. the night before.

TABLE 2

Effect of In-plant Chlorination on Total Plate Counts: Pears and Fruit Cocktail [f]

Date of Expt.	Average in-plant chlorination levels	Average Plate Counts, Bacteria per ml.[g]			
		Pecler spray wash water	Shaker spray run-off, water from peeled fruits	End of flume after dicers	Canned fruit cocktail
	p.p.m.				
1 (Sunday)	....	(Plant not operating but given thorough clean-up)			
2	0.0	410,000	148,000	114,000	86,000
3	0.0	38,024,000	241,000	147,500	84,000
4	0.0	64,526,000	1,236,000	134,078,000	230,450,500
5	4.5 [h]	547,000	574,000	88,000	77,000
6	3.5	134,500	405,000	67,500	71,000
7	4.0	400,000	210,000	354,000	284,000
8 (Sunday)	....	(Plant not operating but given thorough clean-up)			
9	5.0	498,000	356,000	16,000	44,000

[f] Run at the same plant as clingstone peaches.

[g] Two plate counts were made at each sampling point each day (except on 2 and 7—when only one was made) and the values given here are averages of these two counts.

[h] Chlorine was turned on at 9 P.M. the night before.

RESULTS AND DISCUSSION OF SLIME SURVEYS

When equipment surfaces are kept moist by product juices and water, slime usually develops unless the surfaces are treated with a germicide or thoroughly cleaned at least daily. The slime is generally considered an indication of inadequate clean-up and may contribute to contamination of the product.

During each phase of the treatment cycle a thorough inspection of the equipment was made for the presence and extent of slime. The following conditions were observed during the survey:

TABLE 3

Effect of In-plant Chlorination on Total Plate Counts: Apricots [i]

Type of pack	Time of day	In-plant chlorine levels	Plate Counts, Bacteria per ml.					
			Washer overflow [j]	Spray washer overflow after lye peeling	Shaker spray washer	Drippings from rubber conveyor belt	Sirup from hand-filled can	Sirup from machine-filled can
		p.p.m.						
	8:00 A.M.	(A night clean-up preceded day's operation.) Operations started with chlorine on						
Whole,[k] peeled	10:30 A.M.	3.0	10,400	2,700	488	1,140,000	20,400	.....
	11:30 A.M.	Chlorine turned off						
	12-1:00 P.M.	Water wash clean-up						
Whole, peeled	3:00 P.M.	0.0	79,400	6,500	1,730	4,100,000	16,000	.....
Halves,[k] unpeeled	10:30 A.M.	3.0	4,000	.....	.....	.....	10,000	6,700
	3:00 P.M.	0.0	93,000	.....	.....	.....	25,000	21,000

[i] Run at the same plant as Royal Anne cherries. [j] A tank washer was used for halves and a spray washer for whole fruit. [k] Run same day on different lines.

1. During the first (chlorination) phase little or no slime formed on equipment or surfaces which received a continuous or frequent application of chlorinated water. Sprays of chlorinated water on the return sides of belts and elevators kept them free from slime. The use of chlorinated water in flumes, immersion washers, and graders, prevented the growth of slime. In general, the plant appeared clean and there were no objectionable odors. Equipment in which water was partially re-used did have some slime growth, mainly above the water level.

2. When the chlorine was turned off, slime started appearing and at the end of the period without chlorination many surfaces were covered with thick slime. Slime was particularly noticeable on bucket elevators, edges of picking or sorting belts, flumes above the water line, dewatering grids or screens, immersion washer surfaces above the water line, slats of squirrel cage washers, floors or catwalks (slippery), size graders and supporting framework of much equipment. In addition, objectionable odors developed in many places, such as gutters, enclosed equipment, and wood floors.

3. When the chlorine was turned on again the slime became dull or brown in color, and on about the second day began to slough off. In one plant it sloughed so badly that the plant was shut down and the equipment cleaned with brushes to remove the "dead" slime so that contamination of the product would not result. When the chlorine had been on for 3 or 4 days, the plants appeared about as they were during the first phase with chlorination. Also, the objectionable odors disappeared and no new slime developed.

QUALITY STUDIES

Experimental packs were made in the laboratory to determine the effects of chlorine on flavor, color, and odor of 26 canned fruits and vegetables. In addition, the effects on ascorbic acid content and pH were investigated for a few of the products.

EXPERIMENTAL METHODS

The packs were made in the laboratory, but in general the preparation procedures were as nearly as possible those used commercially. Brines and sirups were added cold, because chlorine may be lost from hot brines or sirups, making it difficult to assure the desired residual concentrations. The brines and sirups were made with distilled water.

A sodium hypochlorite solution served as the source of chlorine in the first series of tests, and gaseous chlorine dissolved in distilled water was used for the remainder. There was no difference in the results obtained with either source. Chlorinated wash water and blanching solutions were prepared just before use, and the chlorine was added to the sirups immediately prior to siruping the cans. Chlorine levels of 2, 5, 10 and 50 p.p.m. were tested with three degrees of treatment as follows: (1), adding chlorine to wash water only, (2), adding chlorine to all but brine or sirup water, and (3) adding chlorine to all water including brines and sirups. A control lot containing no chlorine was also included. Six cans were packed for each condition. Three cans were opened three days after canning, and the others six months after canning.

The observations on flavor were made by a panel of five persons trained to recognize the off-flavor peculiar to chlorine in fruits and vegetables. This flavor was classified as medicinal by most of the samplers. Color and odor were also rated by this group.

A glass-electrode pH meter was used to determine the pH of the brine, sirup or juice. The Brix of the sirup was measured by refractometer. The ascorbic acid content of the apricots, peaches and tomatoes was determined by the titration method of Bessey and King *(2)*, and the ascorbic acid content of asparagus and strawberries by the photometer method of Loeffler and Ponting *(7)*. A composite sample from three cans was used for each ascorbic acid, pH, and Brix determination.

RESULTS OF QUALITY STUDIES

The data for the flavor tests are given in Table 4 and represent the worst condition observed in any of the two or three packs of either cutting. There was no noticeable difference between samples cut three days and six months after packing.

Except in the case of Rome Beauty apples, no off-flavors resulted when 10 p.p.m. of chlorine was used in all wash or blanch water, but was not added directly to the canned product. When 5 p.p.m. of chlorine was added to the brine and sirup, or directly to the canned food, off-flavors resulted in apples, figs, clingstone peaches, pears, strawberries, vegetable juice cocktail and yams.

TABLE 4

Effect of Chlorine Treatment on Flavor of Canned Foods

Product	Lowest concentration which produced off-flavor when 2, 5, 10 and 50 p.p.m. of chlorine were added	
	Partial treatment: Chlorination of all water except brines and sirups	Complete treatment: Chlorination of all water including brines or sirups
	Chlorine, p.p.m.	*Chlorine, p.p.m.*
Apple sauce, Rome Beauty [1]	10	5
Apple sauce, Gravenstein [1]	(None at 50)	10
Apricots, halves unpeeled	(None at 50)	50
Apricots, whole peeled	(None at 50)	50
Asparagus, all green	50	50
Beans, green cut	50	10
Beans, green limas	50	10
Beans with pork (recanned) [1]	....	50
Beets, red sliced	50	10
Carrots, sliced	(None at 50)	10
Carrots, pureed [1]	(None at 50)	50
Cherries, Royal Anne	(None at 50)	50
Corn	....	(None with 15) [m]
Figs, whole Kadota	50	5
Grapefruit juice (recanned) [1]	....	50
Orange juice (recanned) [1]	....	50
Peaches, clingstone halves	(None at 50)	5
Peaches, Elberta halves	(None at 50)	10
Peas	....	(None with 10) [m]
Pears	50	2 to 5
Pineapple juice (recanned) [1]	....	10
Potatoes, sweet, solid pack [1]	(None at 50)	50
Pumpkin, solid pack [1]	(None at 50)	50
Prunes, Italian	(None at 50)	10
Spinch	50	10
Strawberries, whole	(None at 50)	5 to 10
Tomato juice [1]	....	10
Vegetable juice cocktail (recanned) [1]	....	5
Yams, sirup pack	....	5

[1] Chlorine added to the product. [m] These tests were made as part of the field studies when the investigation was first begun.

The results, therefore, indicate that no off-flavors should occur in the products tested when the recommended level of 4-5 p.p.m. of chlorine is maintained in the plant water, provided that unchlorinated water is used for making sirups.

Some members of the sampling panel thought a slight odor accompanied the off-flavors; however, this was not considered particularly significant. With apples and pears the addition of 50 p.p.m. of chlorine to the sirup or pureed product produced a slight darkening, but no off-colors resulted from any of the lower concentrations, indicating that chlorine in levels normally used does not have a significant effect on color.

The pH and ascorbic acid content of five products canned with varying amounts of chlorine are given in Table 5.

TABLE 5

Effect of Chlorine on pH and Ascorbic Acid Content of Canned Foods [a]

Product	Degrees Brix	Chlorine concentration, p.p.m.[b]	pH	Ascorbic acid mg./100 g.
Apricot halves	22.2	0.0	3.86	9.1
Apricot halves	19.2	2.0	3.88	9.1
Apricot halves	19.0	5.0	3.88	9.6
Apricot halves	19.2	10.0	3.88	9.4
Apricot halves	20.2	50.0	3.86	8.9
Asparagus, all green	....	0.0	5.56	8.9
Asparagus, all green	....	2.0	5.63	7.6
Asparagus, all green	....	5.0	5.64	7.1
Asparagus, all green	....	10.0	5.62	7.5
Asparagus, all green	....	50.0	5.57	8.8
Clingstone Peaches	21.6	0.0	4.04	5.7
Clingstone Peaches	21.6	2.0	3.97	5.7
Clingstone Peaches	22.8	5.0	4.06	5.8
Clingstone Peaches	20.8	10.0	3.93	6.2
Clingstone Peaches	20.6	50.0	4.09	5.8
Strawberries	....	0.0	3.73	26.8
Strawberries	....	2.0	3.60	25.1
Strawberries	....	5.0	3.63	24.3
Strawberries	....	10.0	3.70	27.8
Strawberries	....	50.0	3.63	25.0
Tomato Juice	....	0.0	4.50	27.8
Tomato Juice	....	2.0	4.48	27.2
Tomato Juice	....	5.0	4.51	27.5
Tomato Juice	....	10.0	4.50	26.8
Tomato Juice	....	50.0	4.51	25.2

[a] Tests made on samples three days after packing.
[b] Chlorine was added to all water used, including brines and sirup.

Chlorine had no noticeable effect on the pH of the products tested. The addition of 50 p.p.m. of chlorine directly to tomato juice caused a slight reduction in ascorbic acid, but there was no recognizable effect when 5 p.p.m. was added.

INDUSTRIAL EXPERIENCES WITH IN-PLANT CHLORINATION

In March, 1950, there were 230 known installations of in-plant chlorination in the U. S. Of these, 165 were in fruit and vegetable canning or freezing plants, and 57 were in fish processing plants. The author has observed in-plant chlorination in canneries packing peas, corn, spinach, asparagus, green beans, lima beans, beets, tomatoes, clingstone peaches, freestone peaches, pears, fruit cocktail, apricots, cherries, pineapple and fish. Some observations made during visits to such plants in 1948 and 1949 are summarized in Table 6.

Most operators are of the opinion that boiler and drinking water should not be chlorinated when high concentrations of chlorine are used for clean-up purposes, and that water for sirups should also not be chlorinated to avoid any possibility of off-flavors.

In the plants visited, chlorination was considered a valuable aid in maintaining plant cleanliness. All but one packer felt it had resulted in considerable reduction in the time required for clean-up. No indications of corrosion attributable to in-plant chlorination were encountered where the recommended levels of 4-5 p.p.m. were maintained for operating periods, and 10-25 p.p.m. for clean-up. In some plants operators stated that less corrosion occurred when chlorine was used, since in their experience most serious corrosion results under slime deposits, and the chlorine had eliminated the slime.

No off-flavors have been reported to date from the use of in-plant chlorination.

CONCLUSIONS AND SUMMARY

1. Lower populations of mesophilic aerobic bacteria occurred in preparation equipment when in-plant chlorination was used than when the water was not chlorinated in canning plants. This was particularly noticeable in equipment where fresh water was constantly added, such as in washers, flumes, etc. Bacterial counts decreased rapidly soon after the chlorine was turned on.

2. A residual chlorine content of 5 p.p.m. was found to be adequte for normal operating conditions. With this concentration there is no danger of off-flavor in the products tested provided that water used for fruit

TABLE 6

Industrial Experience With In-plant Chlorination

Plant	Chlorine levels used [p]		Water not chlorinated	Do plant personnel consider it of value?	Does it save time on clean-up?	Difficulties encountered?	Intend to continue chlorination?
	Daily operations	Daily cleanup					
Vegetables [q]							
	p.p.m.	p.p.m.					
A	5 to 10	40	Drinking	Yes	Yes	None	Yes
B and C	2.5	10	Boiler	Yes	Yes	None	Yes
D	2.5	"High"	Boiler	Yes	Yes	None	Yes
E	5	15	.....	Yes	Yes	None	Yes
F and G	5	10	Boiler, brine	Yes	Yes	None	Yes
H	0.5	2	None	Yes	Yes	Yes [s]	Yes
I	5	10 plus	None	Yes	Yes	None	Yes
J	2.5 to 5	30 to 50	Brine, drinking	Yes	Yes	None	Yes
K	5	"High"	None	Yes	Yes	None	Yes
L	5 to 10	"Wide open"	Drinking, hot water	Yes	Yes	Yes [t]	Yes
M, N, O	2.5 to 5	15 to 25	Drinking, boiler, brine	Yes	Yes	None	Yes
P	3	"On full"	Boilers	Yes	Yes	None	Yes
Q	2 to 5	2 to 5	None	Yes	Yes	None	Yes
Fruits [r]							
R	5	15	Sirup	Yes	Yes	None	Yes
S	2.5	2.5	Sirup, drinking	Yes	(Not known)	None	Yes
T	3.5 to 5	3.5 to 5	Sirup	Yes	Yes	None	Yes
U	3 to 5	3 to 5	Sirup	Yes	Yes	None	Yes
V	About 5	About 5	Sirup	Yes	Yes	None	Yes

[p] Chlorine gas used in all plants except E ,where hypochlorite was used. [q] Vegetables packed were peas, corn, snap beans, lima beans, red beets, and tomatoes. [r] Fruits packed were clingstone peaches, freestone peaches, pears, fruit cocktail, apricots, cherries, pineapple. [s] Personnel believe high chlorine concentrations in blanch water tend to bleach lima beans. Plan to install separate water line for blanchers. [t] Corrosion 5-10 percent above normal. Chlorine levels used were higher than recommended.

sirups is not chlorinated, and phenol or cresol-containing materials are eliminated from contact with the food or water.

3. In-plant chlorination prevented the formation of slime on floors, in gutters, around closing machines, etc., and eliminated plant odors. Slime was also prevented by spraying chlorinated water on the return side of conveyor systems, such as belts and bucket elevators, and by using chlorinated water in flumes and washers.

4. A concentration of 50 p.p.m. chlorine produced off-flavors in several foods, and caused darkening of light fruits such as apples and pears.

5. Chlorine in the residual concentrations normally used (4-5 p.p.m.) had no detectable effect on the pH or ascorbic acid content of the products tested.

6. Plant operating personnel contacted during the surveys considered in-plant chlorination to be a valuable aid to sanitation, and an effective means of reducing the time required for clean-up.

7. No increased corrosion was attributed to chlorination where a residual of 5 p.p.m. was maintained during operations, and 20 p.p.m. for clean-up.

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Composition of Red Raspberries, Including Pectin Characterization[a]

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A study of the chemical composition of several varieties of red raspberries, with special reference to pectin and pectinesterase, was made to determine whether canning characteristics, specifically the tendency to "mush" during processing, might be related to differences in composition. Leading commercial varieties of red raspberries including Cuthbert, Washington, Lloyd George, Willamette, Tahoma, and Newburgh were included in the study. Although the Cuthbert variety does have a higher sugar content and a higher sugar-acid ratio than the other varieties and a lower methoxyl and uronic anhydride content for its pectin, the overall results do not show any direct relation of chemical composition to canning properties. Appreciable amounts of pectinesterase have been found in these berries.

INTRODUCTION

One of the problems in the canning of red raspberries is the separation of drupelets during processing, which results in a "mushy" product with many broken berries. This is largely a varietal problem, as the Cuthbert, for example, shows little of this defect. The Washington variety, a very popular introduction by the Western Washington Experiment Station, tends to "mush" badly during processing. A study of the chemical composition of several varieties of raspberries was undertaken to determine whether canning characteristics are related to differences in chemical composition.

When fruit is bruised so as to release the enzyme pectinesterase, demethoxylation of pectin present in the fruit occurs, with eventual formation of low-ester pectinic acids or of pectic acid. If there are enough calcium or magnesium ions present, a gel consisting of calcium or magnesium pectate or acid pectinate, or mixtures of these, is formed. For this reason, emphasis was placed on pectic substances, which were isolated from several of the leading red raspberry varieties of the Pacific Northwest and subsequently characterized. The activities of the enzyme pectinesterase in the fruit were also determined. In addition, total solids, seeds (number and weight), pH, total acidity, sugars, calcium, magnesium, and crude fiber were also determined.

The constituents generally considered in raspberry analyses are total solids, sugar, and acidity *(3, 9, 16, 23, 25, 33, 34, 36)*. In one article *(20)*, comparing several varieties of Scotch and English berries, the conclusion is reached that a longer growing season and more sunshine result in higher total solids and more sugars and other substances produced by photosynthesis. Three further references deal with the effects of variety and locality *(14, 26, 28)*. Some of the more comprehensive reports on composition of raspberry juice have been included in the bibliography *(4, 8, 11, 30, 31, 32, 35)*.

[a] Report of a study made under the Research and Marketing Act of 1946.

Many investigators have reported the total pectin content of raspberries *(5, 16, 17, 21, 23, 26, 29)*, but only a few have looked for any correlation between pectin content and texture. Rendle *(29)* states that unless the enzymes present have been heat-inactivated, raspberries stored at temperatures ranging from —12 to 37° C. (10 to 99° F.) gradually lose their ability to impart strength to jellies, the rate of loss being more rapid at the higher storage temperatures. There is also a marked loss in soluble and total pectin in the berries when stored for six days at room temperature. Morris *(24)* states that the pectin of raspberries loses 15-25 percent of its setting power in four months' storage at —10° C. (14° F.). Macara *(21)* reports that, if bruised, raspberries will sometimes lose all soluble pectin within 12 hours, and that the product formed as the result of enzyme action is a calcium salt of pectic acid. Macara also states that the enzyme responsible can be destroyed by heating the fruit to 65.6° C. (150° F.). Rendle employed a higher temperature, 82.2° C. (180° F.). One reference *(10)* indicates the composition of a sample of raspberry pectin to be 75.7 percent galacturonic acid, 11.2 percent methoxyl groups, and 0.42 percent ash.

In the present work, chemical analyses were made of six varieties, involving two growing areas, Vancouver and Puyallup, in Washington, and two seasons, 1948 and 1949. The varieties were Washington, Newburgh, Cuthbert, Willamette, Tahoma, and Lloyd George. The Puyallup berries came from commercial plantings, the Vancouver berries from experimental plantings of the Southwestern Washington Agricultural Experiment Station. They were picked early in the morning and shipped promptly to Albany, California, by air express. They were then held at 0.6° C. (33° F.) overnight until they could be analyzed. Ripe but not overmature berries were selected. They were not washed. Analyses were made in duplicate, and average values reported.

ANALYTICAL METHODS AND PROCEDURE

The analyical work (Tables 1 and 2) centered largely on pectin, since it was believed that differences in pectin content or characteristics would probably be associated with texture. Total pectin was determined on one sample of each variety, and moisture, ash, methoxyl, uronic anhydride, and intrinsic viscosity were determined on the pectin isolated from a second sample of the same lot of berries. It should be borne in mind that the knowledge of methoxyl content is of value to indicate the type of pectin in the sample, or the degree to which it has been de-esterified to pectic acid. The uronic anhydride content of the pectin analyzed is an indication of the purity of the isolated pectin. Low methoxyl content may actually be a result of lower purity of the isolated pectin and not of differences in type of pectin. Intrinsic viscosity is correlated with gel strength and also with chain length and molecular weight *(27)*.

Total pectin was determined by the Carré-Haynes method *(2)*.

For the determinations of methoxyl content, uronic anhydride, and intrinsic viscosity, pectin was extracted by a method developed by A. D. Shepherd of this Laboratory. Three gallons of 95 percent ethyl alcohol were heated to 80° C. (176° F.) in a 10-gallon stainless-steel water-jacketed kettle. Ten pounds of berries were introduced. After temperature had reached 75° C. (167° F.), it was maintained at 75-80° C. (167-176° F.) for eight minutes. After the liquor was drained off through cheesecloth, the berries were leached with two gallons of 95 percent alcohol at room temperature, then filtered again. The drained berries were spread on aluminum trays and dried overnight at 60° C. (140° F.) in a vacuum oven. By this treatment the sugars, pigments, and other alcohol-soluble matter were largely removed and the residual pectin thus concentrated.

This dried-blanched material was soaked in about ten times its weight of dilute HCl (pH 1.5-2.0) for 72 hours and filtered through cheesecloth. The filtrate was then treated with a diatomaceous silica filter-aid and filtered through canvas on a Büchner funnel to obtain a clear filtrate for later pectin precipitation. A portion was tested for starch, with iodine, and in each case no starch was indicated.

The clear filtrate was poured slowly into two to three times its volume of ethyl alcohol adjusted to pH 1.0 with HCl. The resulting gel was filtered through cheesecloth, washed twice with alcohol adjusted to pH 1.0 with HCl, and then with neutral 50-65 percent alcohol until chloride-free. To insure thoroughness and to expedite the washing, it was necessary to transfer the gel to a large beaker for many of the washings. The pectin, now with only a slight color due to residual pigment, was hardened by washing with 95 percent alcohol, spread in a thin layer, and allowed to air-dry for about 5 days. The method of isolation as outlined does not extract all the pectin, but probably results in a less degraded product than those extracted by more drastic methods.

For analysis, the air-dry pectin was ground to pass a 40-mesh sieve. Since analytical results were desired on a moisture-and-ash-free basis, moisture was determined by drying 18 hours *in vacuo* at 60° C. (140° F.); ash was determined by heating three hours in a muffle furnace at 600° C. (1112° F.). All the pectin preparations isolated as outlined were remarkably free of inorganic impurities, ash never being higher than 0.4 percent.

Methoxyl content was determined by the saponification method, as developed by von Fellenberg *(7)* and Ehrlich and Kosmahly *(6)*. Details, including modifications in use at this Laboratory, follow: A 0.5 g. sample of pectin was thoroughly wetted with 2-3 ml. alcohol and 100 ml. distilled water. The mixture was brought to pH 7.5 with 0.1 *N* NaOH, using phenol red indicator. Then 25 ml. 0.25 *N* NaOH was added and the mixture allowed to stand for 30 minutes in a stoppered 500-ml. flask. An exactly equivalent amount of 0.25 *N* HCl was then added and the mixture shaken well to dissolve the separated pectic acid. Finally the mixture was titrated to the phenol red point with 0.1 *N* NaOH. The NaOH used in this later titration is a measure of the methoxyl content.

Uronic anhydride was estimated by the method of McCready, Swenson, and Maclay *(18)*, with some slight modifications of the published method, such as the use of a fiber-glass heating mantle instead of an oil bath and the use of a tall glass cylinder as absorber.

Intrinsic viscosity was determined by the method developed by Owens, et al. *(27)*. In making the solutions, 50-55 ml. of distilled water was first added to the pectin (0.1 or 0.2 g.) and the mixture stirred about one-half hour with a mechanical stirrer. Next the pH was adjusted to 6.0. Then a solution of Calgon (1 g.) and NaCl (0.6 g.) was added slowly to this pectin-water mixture, stirring with the mechanical stirrer until no discrete particles of pectin remained (additional 0.5-4 hours). The mixture was then made up to 100 ml. and clarified in a table-model laboratory centrifuge.

Pectinesterase was determined by the method of MacDonnell et al. *(22)*. It was determined on the 1949 samples only. The samples were stored in sealed cans for eight months at —23.3° C. (—10° F.) before analysis. Pectinesterase has been shown to be stable under these conditions *(15)*.

For total solids determinations, a sample of about 20 g. of berries, homogenized in a blender, was weighed into a tared aluminum dish containing about 10 g. of ignited sand, dried (to apparent dryness) about three hours on a steam bath, then 24 hours at 60° C. (140° F.) in a vacuum oven.

The number of seeds per berry, number of seeds per gram, percentage by weight of the berries, and average weight of seeds per berry were determined. This involved a berry count of a given weight of berries, then separation and drying of seeds and determination of the number and weight of the seeds. Representative samples of sound whole berries were selected for

seed count. About 150 g. of berries were stored frozen for each sample until the seed count could be made. The sample was then thawed. About 50 g. (weighed to nearest 0.01 g.) were selected for berry count, and subsequent seed count and weight, following the A.O.A.C. tentative method *(1)*.

The pH was determined on homogenized berries.

For total acidity, a 15-g. sample of homogenized berries was made to 250 ml. in a volumetric flask with CO_2-free distilled water. This was then filtered and a 100 ml. aliquot of the filtrate taken for titration with 0.1 *N* NaOH to pH 7.5.

For the sugar determinations, 1200 g. of berries were blended with an equal weight of 95-percent ethyl alcohol. Samples were removed, and total and reducing sugars were then determined by the alkaline ferricyanide reduction method of Hassid *(12, 13)*.

Calcium was determined as oxalate volumetrically, magnesium as pyrophosphate gravimetrically.

Crude fiber was determined by the A.O.A.C. method, applied to whole berries dried in a vacuum oven for 24 hours at 50° C. (122° F.) then ground to pass a 40-mesh screen.

RESULTS AND DISCUSSION

Resuls of chemical analyses other than those pertaining to pectins and pectinesterase are given in Table 1. Although there are differences in composition which seem to be varietal, such as the high acidity of Newburgh, high sugar content of Washington and Cuthbert, and high sugar-acid ratio of Cuthbert, these do not appear to be related to canning characteristics (mushiness). It will be noted that sugar-acid ratios and total sugars were generally higher in 1949 than in 1948, possibly as a result of seasonal differences, since the growing season in 1949 was quite warm and dry, while in 1948, it was much cooler with considerably more rainfall. Another feature of interest is the higher sugar content of the Vancouver samples as compared with Puyallup, which may be related to the longer growing season at Vancouver. The high crude fiber content of the Newburgh variety, as compared to the others, is interesting, but there are insufficient data to indicate that this is a varietal characteristic.

Table 2 shows that the Cuthbert variety has a lower methoxyl content and a lower uronic anhydride content than any other variety. Since methoxyl content is dependent on the purity of the isolated pectin, the methoxyl values have been reported on the uronic anhydride basis, as well as on the basis of moisture and ash-free isolated pectin.

From the determinations of methoxyl contents and uronic anhydride contents of the isolated raspberry pectins, it is possible to estimate the "purity" of these pectins. In the case of the purest lemon pectic acid isolated at this Laboratory, the total unaccounted-for material was about 8 percent, according to McCready et al. *(19)*. For the Cuthbert raspberry, the total unaccounted-for material averaged 35.3 percent, consistently higher than for the other raspberry varieties tested (averages of 26.8, 25.1, and 24.5 percent for Newburgh, Washington, and Willamette varieties, respectively).

Pectinesterase has been found in all three varieties of berries tested, in somewhat lower activity in Newburgh than in Washington or Cuthbert. The pectinesterase activity of the berries is about $\frac{1}{20}$ of that found in Navel orange flavedo (generally considered a rich source of that enzyme), or about $\frac{1}{4}$ of that found in lemon albedo, or roughly comparable with the activity in whole alfalfa *(22)*. Hence the proportion of pectinesterase in raspberries, although small, is certainly not negligible.

SUMMARY

Analytical data, with special reference to characterization of isolated pectin, have been presented for six varieties of red raspberries, four of which have been sampled in two seasons and three of which have been sampled in two localities. The Cuthbert variety has a higher sugar content and a higher sugar-acid ratio than the other varieties, and a lower methoxyl and uronic anhydride content for its pectin. However, no analytical method was found to consistently predict the "mushing tendency" of raspberries. Pectinesterase has been found in significant amounts in all three varieties of raspberries tested.

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TABLE 1

Chemical Analysis of Red Raspberries, as Received [b]

Year	Variety	Locality	Total solids, %	No. of		Seeds, %	Av. Wt. seeds per berry, g.	pH	Total Acidity		Sugars (as dextrose)		Ratio Reduc. Total Sugars	Ratio Total Sugars: Acid	Ca, %	Mg, %	Crude fiber, on moisture-free basis, %
				Seeds per berry	Seeds per g.				As citric acid,%	Meq. per g.	Reducing, %	Total, %					
1948	Washington	Puyallup	14.2	68.6	24.0	3.84	0.110	3.06	1.61	0.252	3.11	6.54	0.48	4.1	0.022	0.021	15.3
1949	Washington	Vancouver	....	68.4	36.2	5.06	0.096	3.09	1.66	0.258	4.32	9.19	0.47	5.5	0.028	0.020	
1949	Washington	Puyallup	....	73.2	34.1	4.18	0.090	3.09	1.84	0.287	3.41	7.03	0.48	3.8	0.024	0.023	
1948	Newburgh	Puyallup	11.8	72.4	22.4	4.17	0.134	3.01	2.26	0.356	2.40	3.12	0.77	1.3	0.024	0.024	20.1
1949	Newburgh	Vancouver	....	85.6	39.0	5.75	0.126	2.98	1.92	0.299	4.81	7.77	0.62	4.0	0.032	0.024	
1949	Newburgh	Puyallup	....	75.2	28.2	4.26	0.109	3.22	1.48	0.230	3.66	5.60	0.65	3.8	0.017	0.019	
1948	Cuthbert	Puyallup	15.4	71.5	31.9	4.17	0.093	3.23	1.17	0.183	4.90	7.49	0.65	6.4	0.021	0.020	15.5
1949	Cuthbert	Vancouver	....	79.3	44.4	5.46	0.097	3.28	1.48	0.230	6.00	9.18	0.65	6.2	0.032	0.024	
1949	Cuthbert	Puyallup	....	80.0	42.6	4.19	0.082	3.29	1.24	0.193	5.38	9.23	0.58	7.4	0.025	0.022	
1948	Willamette	Puyallup	10.7	86.0	19.5	3.07	0.135	3.25	1.98	0.309	1.98	2.79	0.71	1.4	0.014	0.024	15.8
1949	Willamette	Puyallup	....	106.8	29.4	3.60	0.130	3.31	1.68	0.262	3.27	5.18	0.63	3.1	0.018	0.025	
1948	Tahoma	Puyallup	11.6	88.2	29.4	3.43	0.103	3.03	2.06	0.321	3.20	3.96	0.81	1.9	0.015	0.020	15.9
1948	Lloyd George	Puyallup	11.9	75.7	24.2	3.56	0.111	3.01	1.87	0.292	3.10	4.34	0.71	2.3	0.015	0.021	14.5

[b] For all analyses, except crude fiber.

TABLE 2

The Pectin and Pectinesterase of Red Raspberries

Year [a]	Variety	Locality	Total pectin, % [d]	Methoxyl in pectin, % [e]	Methoxyl in pectin, % [f]	Uronic anhydride in pectin, % [e]	Intrinsic viscosity of pectin [e]	Pectinesterase [PE. u]g. [g]
1948	Washington	Puyallup	.....	9.9	13.4	73.8	2.5	.....
1949	Washington	Vancouver	0.86	9.4	13.8	68.2	3.4	.0027
1949	Washington	Puyallup	0.87	9.4	13.5	69.8	2.6	.0030
1948	Newburgh	Puyallup	.....	8.5	13.0	65.6	1.1	.....
1949	Newburgh	Vancouver	0.99	10.0	14.0	71.2	3.0	.....
1949	Newburgh	Puyallup	0.68	9.2	13.1	70.4	2.2	.0012
1948	Cuthbert	Puyallup	.....	8.4	13.4	62.6	2.9	.....
1949	Cuthbert	Vancouver	0.94	7.6	13.1	57.8	1.5	.....
1949	Cuthbert	Puyallup	0.97	7.4	11.7	63.3	1.2	.0029
1948	Willamette	Puyallup	.....	10.0	14.0	71.2	3.6	.....
1949	Willamette	Puyallup	0.77	8.4	11.8	71.4	4.2	.....

[a] The 1948 results above were on stored, frozen material; the 1949 results were on fresh berries. [d] As received. [e] On moisture-and-ash-free basis on the isolated pectins. [f] Parts per 100 parts of uronic anhydride. [g] Milliequivalents of methyl ester linkages of pectin hydrolyzed per minute per g. of berries as received.

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Technology and Utilization of Gelatin[a]

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A REVIEW

Production methods are outlined for manufacturing gelatin from acid treated and lime cured raw materials. Major uses in food and drug industries are discussed.

When the individual who has no direct contact with the gelatin industry thinks of gelatin he thinks of it as a definite material with unvarying qualities. He does not realize that it is prepared with wide ranges of physical characteristics. For instance, gel strength varies from products with practically no gel strength to those with maximum gel strength. Viscosity also varies over a wide range.

Advantage is taken of the variation in characteristics of gelatins to select the type which is most suited for each application. In some cases where gelatin has been tried for a specific purpose, the results have not been as desired. The failure may have been because gelatin of the right characteristics for that application was not used.

The gelatin manufacturer can to a large extent control his operation to obtain the type of gelatin desired for each use. However, gelatin is prepared from biological raw materials and as would be expected, there is variation from one cooking to the next. Therefore, it is necessary to blend portions of tested lots in order to maintain uniformity for each gel strength grade.

Gelatin is made by a controlled hydrolysis of collagen. Collagen is a protein which occurs in many animal tissues. In muscle it contributes to the characteristic texture of meat. Major factor in aging process of meat is attributed to enzyme breakdown of collagen fibers. Collagen fibers are referred to frequently as white connective tissue.

Tissues containing the highest amounts of collagen are skin, bones, sinews and gut. At this point, we wish to emphasize that hooves and horns are principally keratin which can not be converted to gelatin. We don't know who is responsible for starting the false story about gelatin being made from these materials, but it has appeared in many publications. In view of the fact that gelatin is a pure food product, we are glad to observe that this fallacy is disappearing from most recent literature.

Tissues of commercial importance in manufacture of gelatin are hides and bones. To be more specific, these include parts of pork skins removed at time of slaughter, beef bones and other portions of carcasses. Pork skins are frozen in molds of approximately 100 pounds each and shipped to the gelatin plant in refrigerator cars. Bones are processed in the packing house to remove adhering meat and then dried.

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PREPARATION OF STOCK FOR COOKING

The most common procedure for treating pork skins is by the so called acid process. The frozen molds are thawed in water followed by washing in and out for several hours. After washing, skins are held in dilute acid until penetrated. Acids used are ordinarily hydrochloric, sulphuric or phosphoric. Skins are then washed with fresh water until of desired acidity for cooking. Stock is then drained and loaded into the cook tanks. Gelatin from stock treated as just described, ordinarily has a pH in the range of 4.0 to 4.8.

Collagen in bone will not readily convert to gelatin after a short acid treatment as do pork skins and requires considerable more treatment.

Dry bones are crushed and then charged into wooden tanks for extraction of minerals. These tanks are arranged in batteries of four. Dilute hydrochloric acid is circulated through the battery in a counter current fashion. That is, the fresh acid is fed into the tank containing the bone which has been longest in the system and contains the lowest mineral content. The last tank in the battery is the one last filled with bone. By this procedure, the greatest economy of acid is obtained. Extraction of minerals is complete when no hard centers are left in bone pieces. After extraction of minerals, the remaining organic material is known as ossein. Collagen fraction in bone is approximately 25 percent on a dry basis.

The acid liquor containing minerals extracted from the bone is treated with lime and phosphate recovered in form of di-calcium phosphate. This product is frequently referred to as precipitated bone.

After removal of minerals, ossein is held in lime steep for several weeks. Time varies with prevailing temperature. That is, the higher the temperature, the more rapid liming progresses. As a rule, however, this operation is carried out at temperatures below approximately 65° F. (18° C.). At higher temperatures, the ossein disintegrates to an excessive extent.

The so called liming operation is carried on in large vats. Stock is covered with milk of lime. The lime slurry is drained and replaced, three to five times during the liming period.

During the liming operation, a large share of the proteins other than collagen are removed. A small amount of collagen is disintegrated, but not to the same extent as the other proteins. Collagen goes through a partial specific hydrolysis during liming *(5, 9)*. Not a great deal is known about the changes in collagen which take place during liming. Ammonia is given off and the isoelectric point shifts from the region of 7 to approximately 5 *(2, 3)*. This shift according to Ames *(6)* is due to loss of ammonia from acid amide groups.

Following the liming process, ossein is washed free of adhering lime and then steeped in acid, ordinarily

sulphuric, phosphoric or hydrochloric. When stock is penetrated, it is washed back until it will cook out at the desired pH. This is at a higher pH than when cooking acid cured pigskins. Gelatin produced from limed stock is commonly referred to as alkaline type.

After stock preparation, remainder of processing is very similar for all type of raw material.

CONVERSION TO GELATIN AND PROCESSING THEREOF

Stock is loaded into open cook tanks and covered with water. Cook tanks are heated by means of steam coils or steam jacket. Bottoms of tanks are provided with perforated false bottoms so that liquors can readily be drained from the stock.

Gelatin is extracted from the stock with successive portions of water at increasing temperatures. First extraction is in the range of 110 to 140° F. (43 to 60° C.), while the last extraction is at boiling temperature. Reason for this system of extraction is to prevent breakdown of the gelatin molecule which increases with rise in temperature *(7)*. Therefore, extraction is carried out at the minimum temperature that will produce a satisfactory yield. As has no doubt been gathered, gel strength decreases with each run. The first run has the highest gel strength and the last one has a very low strength.

Liquors drained from the cook tanks are filtered through pressure filters. Diatomaceous earth and activated char are commonly added as filter aids.

Gelatin content in liquors from the cook tank is in the range of 3 to 7 percent. In most cases, the liquors are put through evaporators where 50 to 75 percent of the water is removed under vacuum.

Evaporated liquors are run onto a continuous rubber belt which passes through a refrigerated tunnel. On this belt, the liquor changes to the gel form. The sheet of gel is cut into appropriate lengths and dropped onto wire nets approximately 30 x 50 inches. Nets are stacked on dollies which are placed in drying tunnels.

Drying is ordinarily carried out in two stages. During the first stage, the dry bulb temperature is approximately 90° F. (32° C.). When moisture content is down to 20 to 30 percent, the stacks of nets are transferred to the so called hot tunnels. Temperature in these tunnels is considerably higher than in the first tunnels. In this final stage of drying, moisture is reduced to approximately 10 percent.

Dried sheets of gelatin are removed from the nets and broken down to the desired particle size. Common practice is to first put the large sheets through a crusher which breaks them into flakes with maximum diameter of about 1½ inches. This is the so called broken flake form. In a second break, product is reduced to approximately 4 mesh: Final grind is to 30 or 40 mesh. Specialized type impact mills are used for grinding dry gelatin. It is important that the moisture content at this period be below approximately 11 percent so that the product will readily shatter in the mill.

TYPE OF EQUIPMENT

In the matter of equipment, the change as in other food manufacture has been to stainless steel. Aluminum and nickel have been used to a great extent in this industry, but when replacement is necessary, it is usually made with stainless steel. Glass lined equipment has been used to some extent and is very satisfactory as long as the film of glass is unbroken.

SANITATION DURING MANUFACTURE

Gelatin is a fairly good medium for the growth of bacteria and, therefore, sanitation is of prime importance. A quarter of a century ago it was felt that to produce gelatin of low bacteria count, for example 1000 or less plate count, a preservative was required. At that time, it had been reported by some investigators that gelatin was a contributing factor to high bacteria counts in ice cream.

Fortunately, it was found that by installation of equipment which could be cleaned and by regular cleaning thereof bacteria counts could be reduced to the desired low level. It had been common practice to have permanent installations of pipes and valves so that a batch of liquor could be transferred from a given point to almost any other point in the plant by opening and closing the proper valves. A man with the title of "Liquor Chaser" followed through on each run to be sure it was transferred through the proper channels. Needless to say, the maize of piping was found to be a contributing factor in high bacteria counts. Eliminating of permanently mounted, cross-over piping and dead ends practically eliminated contamination from piping. Biggest source of gelatin liquefying bacteria was found to be in wooden equipment. Replacement of wooden receiving and storage tanks with metal ones materially reduced the numbers of these organisms.

In addition to thorough and frequent cleaning of process equipment, rapid handling of liquors is necessary to keep bacteria at a low figure. It is interesting that in drying gelatin from the gel state, the bacteria counts decrease more often than they increase. This in spite of the fact that 12 to 20 hours are required for this operation. Our theory on this phenomena is that because of the constantly changing concentration of the medium, bacteria do not acclimate themselves and can not get started on their growth cycle.

Gelatin, like other food products, must meet rigid standards for heavy metals. Meeting these standards influences the selection of processing equipment and requires careful checking of all materials added during processing. These materials include lime, acids, diatomaceous earth and activated carbon.

GELATIN TESTING

In addition to tests such as bacteria counts, trace metal analysis, pH, moisture and ash, gelatins are put through tests which indicate their working qualities.

Most important consideration in evaluation of gelatin is the gel strength. This is determined by use of the Bloom Gelometer. The test consists essentially of preparation of a 6⅔ percent solution, holding for 17 hours at 50° F. (10° C.) and testing on the Bloom Gelometer. This apparatus measures weight of shot required to force a ½ inch round plunger four millimeters into the gel. Values obtained on gelatin range from 0 to slightly over 300 grams.

Viscosity is of importance for many uses and is determined at 6⅔ percent concentration at 140° F. (60° C.). The standard test consists of measuring the time of outflow from a pipette of approximately 100 cc. volume. Each pipette is calibrated and by reference to the chart for that particular pipette, time in seconds of outflow is converted to millipoises of viscosity.

Gel strength as determined by the Bloom Gelometer and the viscosity measurement give only a partial indication of the working qualities of a particular gelatin. The product for a particular use is quite often put through a test which will approximate the conditions of the use. For instance, a gelatin for gelatin dessert is tried out in a formula approximating commercial practice. It is then tested for quick set and overnight strength under controlled conditions. Gelatins for marshmallow are tried out in a marshmallow formula in a small size beater.

USE OF GELATIN

As indicated in the discussion of testing methods, value of gelatin is dependent primarily upon its physical qualities. The most valuable property being that of formation of a reversible gel.

The greatest single use of gelatin is in gelatin dessert, which is one of America's favorite desserts, particularly for children. In this use, the gelatin is blended with sugar, an organic acid such as citric or tartaric, flavor and color. Gelatin percentage in the dry mix is on the order of 7 to 10 percent. Correct amount of gelatin to use varies inversely with the basic gel strength of the gelatin. That is, with a lower Bloom strength an increased amount is required.

In gelatin desserts, a quick set is desirable. It is also desirable that maximum gel strength not cause the product to be rubbery. The product should also not melt down too rapidly at room temperature. A gelatin of relatively high gel strength when adjusted to optimum concentration will more nearly meet these requirements than one of low gel strength.

A gelatin of 100 Bloom and one of 250 Bloom can be adjusted in amounts in gelatin dessert so both show the same gel strength at 50° F. (10° C.). However, if the gels are held at 75° F. (24° C.), the one prepared from the gelatin with lower Bloom strength will start to melt within a few minutes.

For gelatin dessert purposes, it is usually recommended that the gelatin have a Bloom strength of 225 or higher.

Gelatins for marshmallow manufacture are selected on the basis of whipping properties and texture of the final product. In making novelties such as imitation ice cream cones, Easter chicks and Halloween faces, a very light nad fluffy marshmallow is desired. A low Bloom gelatin of 75 to 125 Bloom most nearly meets the requirements. The presence of peptones in the partially hydrolized gelatin causes a faster whip and produces a more fluffy marshmallow.

Opinion varies considerably as to which is the best type gelatin to use, for marshmallow deposited on cookies. A fairly low density marshmallow is desired, that is, around 40 ounces per gallon. Marshmallow should be fairly resilient, but tender when bitten into. Some manufacturers use a low Bloom gelatin in the winter time and then switch to a higher gel strength in the summer in order to compensate for a greater melting tendency at higher temperatures. As a rule, gelatins selected for deposit on cookies are in the range of 175 to 225 Bloom.

The manufacturer of the ordinary white cast candy marshmallow has the problem of making a product which will retain some semblance of shape in flexible cellophane bags even when piled several packages high in the retail store. What is desired is maximum resiliency without extreme toughness. A gelatin of around 225 Bloom is usually used for this purpose. Marshmallows prepared with the 100 Bloom gelatin which works best for the novelty manufacturer would produce marshmallow which would not retain shape under the pressure present in cellophane bags.

In many cases, the most suitable gelatin is found by collaboration between the gelatin manufacturer and the plant making marshmallow.

In the dairy industry, the principal use of gelatin is as a stabilizer in ice cream. A relatively small amount of gelatin prevents growth of large ice crystals and imparts other desirable characteristics to ice cream which in our opinion are not duplicated by use of other stabilizers.

Gelatin of any Bloom strength will effectively stabilize ice cream. The amount to use varies inversely with the gel strength. As a rule, it is slightly more economical to use the higher Bloom strengths.

In the packing house, considerable gelatin is used for coating specialty meat products and in canned meats to absorb juices which separate during the cooking processes.

In the pharmaceutical industry, the greatest amount of gelatin is used for making capsules. For the so called elastic or soft capsules, a medium Bloom strength gelatin works to best advantage. For hard capsules, which are made in two parts, relatively high gel strength gelatin is used.

Selected non-pyrogenic runs of gelatin have been used successfully as a blood substitute in treatment of shock. Indications are that gelatin is eventually metabolized completely and does not segregate in any vital organs as do some of the other materials which have been tried out as plasma substitutes.

In the preparation of pharmaceutical emulsions, the isoelectric point of the gelatin must be taken into account *(8)*. In the U. S. Pharmacopea, reference is made to Pharmagel A and Pharmagel B. The A type is gelatin prepared by acid treatment of the original stock and has an isoelectric point of approximately 8 pH. The B type is prepared from alkali cured raw materials and has an isoelectric point in the region of 4.7 to 5.0 pH. With the A type, greatest stability is imparted to an oil-in-water emulsion in the range of 3 to 4 pH. With Pharmagel B, it is recommended that the pH be adjusted to approximately 8.

When preparing emulsions containing both gelatin and vegetable gums, the charges on the two materials must be taken into account. That is, if one carries a negative charge and the other a positive charge, a pre-

cipitation may take place. This can usually be taken care of by replacing the gelatin with one having the other isoelectric point. As an example, we have a mixture of 5.5 pH containing gelatin and irish moss. The so-called acid-cooked gelatin with isoelectric point around 8 will precipitate with irish moss while gelatin from a limed precursor with isoelectric point of about 5 will not. This phenomena is also of importance when a wetting agent is added to a solution containing gelatin, particularly if the wetting agent is anionic or cationic. Assuming again a pH of 5.5 to 6.0, a cationic wetting agent will tend to precipitate with the gelatin having an isoelectric point at 5.0, but not with the other type gelatin. With anionic wetting agents, the reverse is true. The safest procedure when using a wetting agent in a gelatin solution is to use one that is nonionic.

Because of its mechanical qualities, gelatin is also used in several products in the graphic arts *(4)*. Its greatest single use in this field is as a carrier for silver halides in emulsions used for photographic film and paper.

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Mass and Infrared Spectra of Apple Vapors

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Collection techniques of low-temperature trapping and adsorption-desorption, and analytical techniques of mass and infra-red spectrometry are examined as tools for more intensive study of the high molecular weight contaminants in apple storage atmospheres. It is shown that gases in apple storage atmospheres include wide ranges of molecular weights and several varieties of functional groups.

INTRODUCTION

The use of air purification by activated carbon in the storage of apples is well known and its effects have been fully described in the recent literature *(7, 8, 9, 10, 12)*. Briefly, these effects include reduction of scald, retardation of ripening effects and control of foreign odors.

The nature of the atmospheric contaminants which are present in apple storages, however, has been only partially investigated. Southwick *(13)* determined acetaldehyde by passing an air stream containing apple vapors through sodium bisulfite solution. Several analytical procedures for determination of ethylene have been developed, of which the method by Gross *(3)* is the most recent. This procedure takes advantage of the fact that ethylene will pass through 95 percent sulfuric acid, which absorbs the other organic volatiles present. The ethylene is then quantitatively absorbed by a solution of magnesium perchlorate in perchloric acid and is determined by a cerate oxidimetric procedure.

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Other work has been done on the analysis of apple components but this work has been done on the fruit itself and not directly on the vapors as they are naturally given off during storage. This includes the important earlier work of Power and Chesnut *(5, 6)* who used the method of steam distillation of apple parings and pulp to collect and identify various amyl esters and geraniol. More recently, White *(15, 16)* has used chromatographic adsorption in the analysis of apple essence produced by flash distillation from fresh ripe apples. He identified eight alcohols, five carbonyl compounds, and nine ester components in the essence. It should be noted, however, that flash distillation may give different materials from those given up by the apples to the atmosphere in normal storage, and this is not, therefore, a frontal attack on our problem.

A more precise knowledge of the nature of apple vapors should be helpful in a number of ways. More information is needed on the mechanism by which activated carbon air purification is effective. Comparisons of the composition of the gases in apple storage with the composition of gases actually adsorbed on the activated carbon will show which of the contaminants are and which are not removed. It may also show whether the gases removed from the storage atmosphere are simply retained on the carbon by physical adsorption or whether they undergo any chemical changes.

If the contaminants in question vary widely in molecular weight, the effect of varied rates of diffusion will be important, since the rate of diffusion is inversely

proportional to the square root of the molecular weight. A recent experiment on the storage of apples in a wind tunnel *(11)* shows that the effect of diffusibility is of great importance in the removal of scald gases. It would, therefore, be very valuable to be able to determine the molecular weights of gases in different portions of the storage space.

The present investigation has been carried out to evaluate the usefulness of mass spectral analyses in attacking the problems described above. It should be clear that a thorough study of these problems requires point-by-point investigation of the many questions involved. A preliminary selection of promising techniques, however, can save considerable later efforts.

MASS SPECTRAL ANALYSIS

The mass spectrograph, first built by Aston and Dempster before 1920, is an instrument which separates atomic and molecular particles according to their masses. Its most important early use was in the separation of isotopes, atoms of the same element which differ in mass. One important use of this instrument in atomic research was the identification of the various uranium isotopes. The same principle, on a much larger scale, was actually used to produce the first large size samples of pure uranium-235, by separating this fissionable material from uranium-238 and other naturally occurring uranium isotopes.

Only within the last few years has this method been used to make precise analyses of more complex gas mixtures. The work along these lines has been directed largely to mixtures of petroleum gases.

The method by which the mass spectrometer effects separation and analysis of a gas mixture includes the following steps:

First, the molecules of the gas mixture, at a very low pressure, are bombarded by a stream of electrons from a hot filament. This strips one or more electrons from the bombarded molecules and gives them a positive charge. Some of the molecules are cracked into smaller fragments and these fragments are similarly charged by the electron bombardment.

Second, the positive ions thus produced are accelerated along a given path between two slits by an applied potential difference. This gives a thin beam of ions in which each particle has the same energy, which may be expressed as

$$eE = \tfrac{1}{2}mv^2$$

where e is the charge on the ion, E is the applied potential, m is the mass of the particle and v is its velocity.

Third, this beam of ions is passed through a uniform magnetic field, which causes the beam to deflect by a force equal to Hev, where H is the magnetic field strength. The centrifugal force counteracting this deflection is mv^2/r where r is the radius of deflection.

Then, $Hev = mv^2/r$ and, substituting the energy value for v in the equation, we obtain $m/e = H^2r^2/2E$.

If the particles are given a unit charge, $e = 1$, the above equation is an expression for M, the mass.

If we solve for Hr and express all the factors in conventional units, $Hr = 144\ MV$, where H is expressed in gauss, r is expressed in cm., M is expressed in atomic weight units, and V is expressed in volts. If H and r are kept constant, then $MV = H^2r^2/144 = K$.

The mass entering the collector is inversely proportional to the voltage. The voltage is varied continuously to bring successively each mass to the collector. The intensity of the current produced by each of the separated beams of ions is recorded automatically to give the mass spectrum.

When a single pure gas such as ethanol vapor, is subjected to the mass spectral analysis, the result obtained shows not only the mass of ethanol (46) but also the masses of the fragments split off in the ionization process. This spectrum, or "cracking pattern," is typical only of ethanol and of no other gas, and its appearance in an analysis definitely shows the presence of this material. Figure 1 shows the mass spectrum of ethanol vapor.

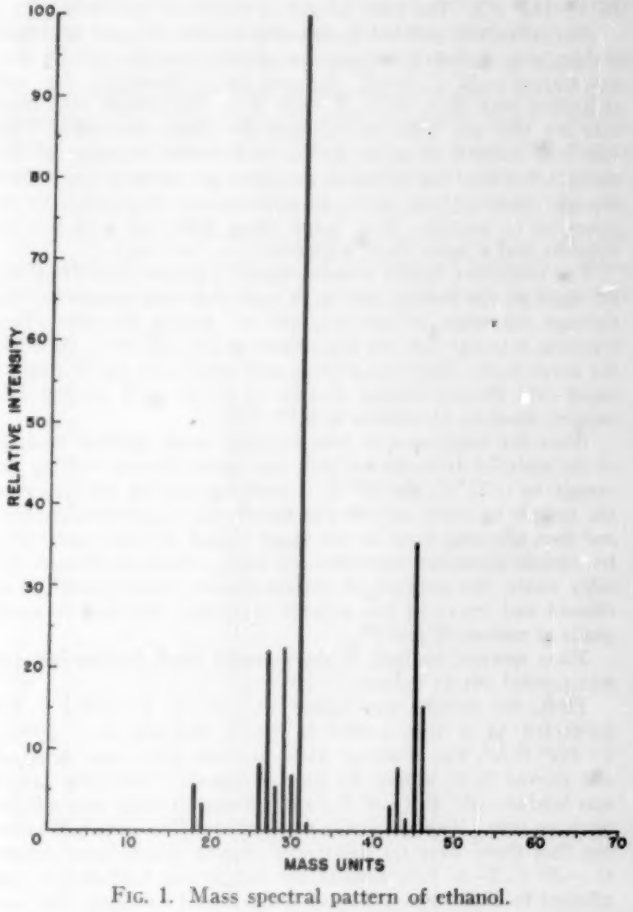

FIG. 1. Mass spectral pattern of ethanol.

When a complex mixture of gases is subjected to analysis, the resulting composite spectrum must be mathematically analyzed to define the individual mass spectral cracking patterns of which it is composed. This assumes some information about the nature of the gases present and a previous knowledge of the cracking patterns of these likely components. This means that a great deal of previous work must be done with the types of mixtures to be investigated before the full benefit of this technique is realized. Such work has already been carried out by the petroleum industry for hydrocarbon gases, and mass spectral analysis is much used in this field. In the case of apple and other food gases, however, the only previous information available consists of the cracking patterns of some alcohols, esters, aldehydes and other oxygenated compounds *(2, 4, 14)*. More specific and extensive background research is needed. The experimental work described below will indicate the types of problems to be faced and results which may be promised from such work.

EXPERIMENTAL

Trapping of Gases From Apple Storage. In an effort to carry out a direct gas analysis of the more easily diffusible components of apple vapor, the following technique was employed.

Duchess of Oldenburg apples were picked on August 12, 1949, and stored at 10° C. (50° F.) until August 15, when the tests were begun. These apples had a firmness of 14.1 according to the Magness-Taylor pressure tester and a ground color of 1 to 2 according to the Cornell color chart. In this and each of the subsequent tests, forty fruits were placed in a five-gallon glass jar immersed in a constant temperature bath at 28° C. (82° F.). The total weight of the fruits was 5876 g.

Air, previously purified by drawing through 80 g. of activated carbon, was aspirated through the apples, then through an ice-salt trap at —10° C. (—23° F.) and finally through a dry ice-cellosolve trap at —75° C. (—103° F.). The initial rate used was one liter per hour, as measured by a ball rotameter. This rate was adopted so as to permit quantitative trapping of the gases. This low rate of aspiration, however, allowed the carbon dioxide content to go up to 12 percent and the oxygen to go down to 11 percent. This could mean abnormal evolution of volatiles and a more rapid aspiration was necessary.

The procedure finally adopted was to aspirate with fresh air all night at the rate of one cu. ft. per hour and to put the air through the traps at one liter per hr. during the day. This trapping was carried out daily from 6:30 A.M. to 6:00 P.M. for seven days. Even this nightly aspiration with air at a fairly rapid rate allowed carbon dioxide to go up to 8 percent and oxygen down to 14 percent by 6:00 P.M.

After the conclusion of this trapping, mass spectral analysis of the material from the ice trap was carried out by chilling the sample to —75° C. (—103° F.), pumping out the air, allowing the sample to warm up to room temperature (pressure 26 cm.) and then allowing some of this vapor to leak into the spectrometer sample container (pressure 2.4 cm.). Analysis showed, besides water, the presence of carbon dioxide, small quantities of ethanol and traces of less volatile materials indicated by small peaks at masses 76 and 77.

Mass spectral analysis of the material from the dry ice trap was carried out as follows:

First, the sample was cooled to —75° C. (—103° F.) and connected to a trap cooled in liquid nitrogen at —195° C. (—319° F.). The material thus distilled over was analyzed and proved to be almost all carbon dioxide. Next, the sample was held at —20° C. (—4° F.) and allowed to distill into a liquid nitrogen trap. No appreciable material was thus obtained, showing that there were no substances present which were volatile at —20° C. (—4° F.). Finally, the sample was held at 0° C. and allowed to distill into a trap cooled in liquid nitrogen. The material thus collected was subjected to analysis and the spectrum obtained is shown in Figure 2.

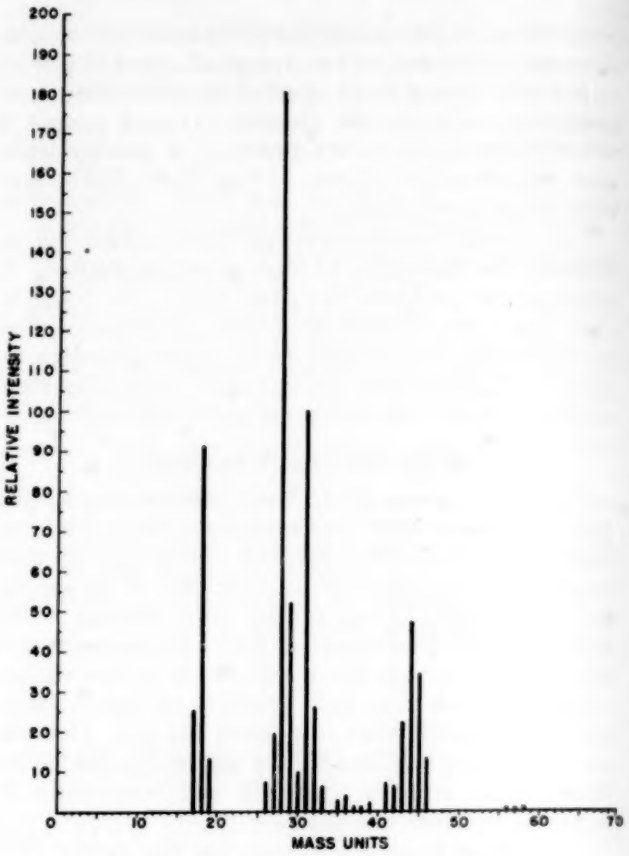

FIG. 2. Mass spectrum of trapped gasses.

An interpretation of Figure 2, using available information, indicates the following:

First, the method of removing vapors from apples by a slow air stream succeeded in separating only the lighter gases, as shown by the fact that the highest mass of reasonable intensity in this spectrum is 46, with additional small pips indicating traces of materials at masses 56 to 58. This substantiates Smock's wind-tunnel experiment mentioned above which showed that some heavier gases are removed only at high rates of air flow.

Second, it is possible with this spectrum to make an analysis of the major components of the trapped material. Since the highest mass with significant intensity indicated is 46, which is the molecular weight of ethanol, we may look for the pattern of ethanol in this spectrum. Assuming that ethanol makes all the contribution to the ion intensity at weight 46, we may recalculate the pattern of ethanol adjusting its relative intensity at weight 46 to 13 and subtract this pattern from the original spectrum. The result of this procedure is shown in Table 1. It will be seen that the entire pattern of ethanol is included in the spectrum of the mixture and, furthermore, that five of the peaks, at masses 26, 27, 31, 45 and 46, are accounted for completely or substantially by the ethanol. This identitfies ethanol as one of the

TABLE 1

Analysis of Spectrum of Trapped Gases

m/e	Relative Intensity		
	Mixture	Ethanol	Residue
17	25	....	25
18	91	5	86
19	13	3	10
20	....	....	....
21	....	....	....
22	....	....	....
23	....	....	....
24	....	....	....
25	....	....	....
26	7	7	0
27	19	19	0
28	180	5	175
29	52	19	33
30	10	5	5
31	100	85	15
32	26	1	25
33	6	....	6
34	....	....	0
35	3	....	3
36	4	....	4
37	1	....	1
38	1	....	1
39	2	....	2
40	....	....	....
41	7	1	6
42	6	3	3
43	22	6	16
44	47	1	46
45	34	30	4
46	13	13	0

trapped gases. The residue after subtracting the contribution of ethanol appears in column 4 of Table 1. The large intensities at masses 18, 28 and 32 may be accounted for by the backgrounds of water, nitrogen and oxygen respectively, which were present to some extent in the apparatus and which appeared in a background spectrum obtained before the run reported here. An examination of the cracking pattern of acetaldehyde (Figure 3), shows that this substance has its major

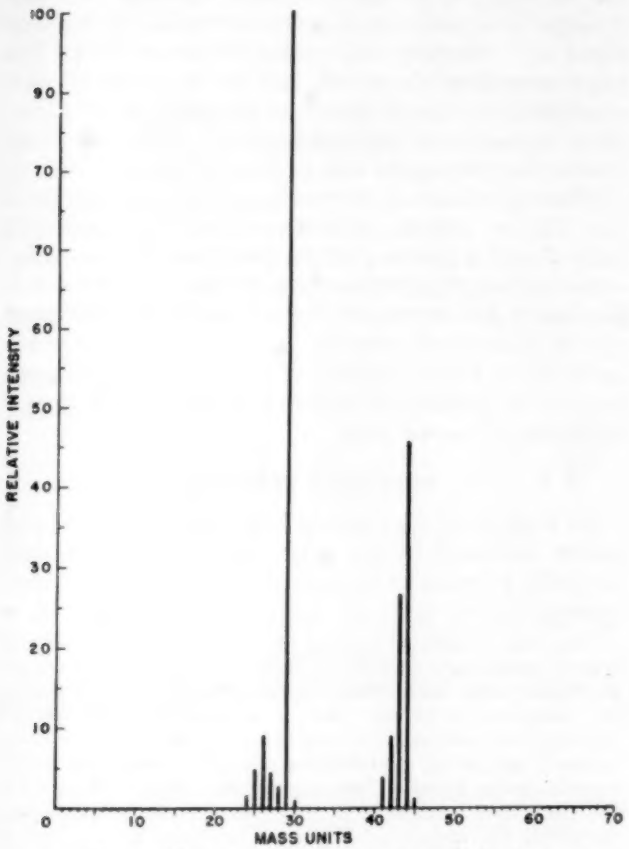

FIG. 3. Mass spectral pattern of acetaldehyde.

peaks at masses 29, 43 and 44. Each of these peaks is prominent in the residue of the spectrum of the mixture in column 4 of Table 1, so that the presence of acetaldehyde in the trapped gas mixture is indicated.

Adsorption of Apple Vapors on Carbon. A second five-gallon glass jar containing 6270 g. of apples (40 fruits) from the same source as the fruit in the previous run was thermostatted as described above. This jar was connected to a 55-gallon gas-tight drum which contained a canister with 355 g. of activated cocoanut shell carbon. The bed thickness of the carbon was ¾" and the recirculated air was drawn through the bed at 10 cu. ft. per m. The rate of flow of air circulating between the fruit and the drum was 1 cu. ft. per hour. Due to the deep reservoir of air in the drum the carbon dioxide went up to only 2 percent and the oxygen content down to 19 percent. Recirculation was continuous. The ratio of weight of carbon to weight of fruit was much greater in this experiment than it is in commercial storage. Air flow was continued for seven days.

The adsorbed gases were then removed for analysis by warming the carbon in a high vacuum and passing the desorbed gases through a train of three traps: ice-salt, dry ice, and liquid air. Practically all of the organic vapors were trapped in the dry ice, and the spectrum of the sample thus obtained appears in Figure 4. The masses here obtained show clearly how a greater rate of air flow permits removal of gases of higher molecular weights. The various prominent peaks observed are typical of specific types of compounds, such as mass 29 for aldehydes, 43 for acetates or methyl ketones, mass 45 for secondary alcohols, etc. More intensive work is required, however, before a spectrum of this complexity can be subjected to exact analysis.

A third five-gallon jar containing 6052 g. of apples as described above was connected to a glass tower containing only 10 g. of activated cocoanut shell carbon. The system had recirculation of air from the apples through this tower at the rate of 1 cu. ft. per hour continuously during the first two days of the test. At this time the carbon dioxide value had gone up to 7 percent and the oxygen down to 14 percent. Thereafter clean air was aspirated over the apples and then through the

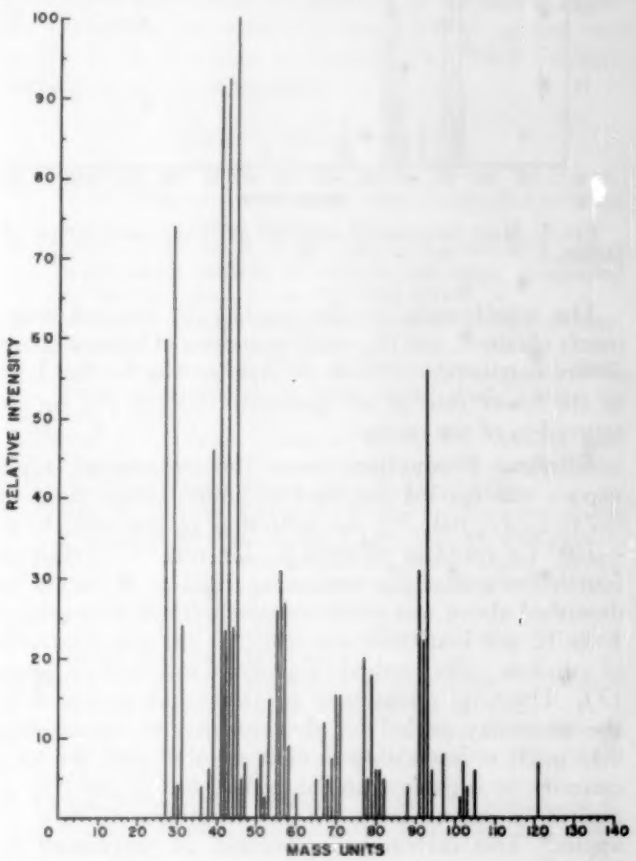

FIG. 4. Mass spectrum of adsorbed gas from large sample of carbon.

tower at night and recirculation was used during the day. The smaller ratio of weight of carbon to weight of apples used in this run approximates the quantities of carbon used in commercial air purification more closely than does the run previously described. The gases from this small sample of carbon were desorbed, trapped, and subjected to mass spectral analysis in the same manner as described for the previous run. The spectrum obtained is shown in Figure 5.

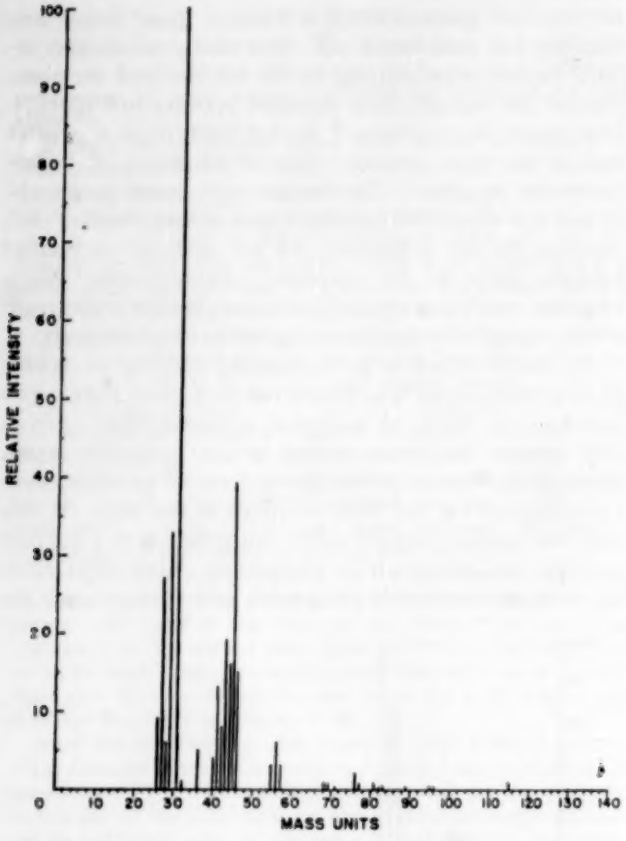

FIG. 5. Mass spectrum of adsorbed gas from small sample of carbon.

The significantly smaller variety of cracked fragments obtained, and the small quantities of masses above 58 are noteworthy. These differences may be due both to the lower rate of air movement and to the earlier saturation of the carbon.

Ethylene Production. Since the trapping of apple vapors was carried out at a minimum temperature of —75° C. (—103° F.) no collection of ethylene, b. p. —104° C., could be effected in this way. Therefore, a fourth five-gallon jar containing 5952 g. of apples as described above was continuously aspirated at a rate of 1 cu. ft. per hour and was used for the determination of ethylene. The method of analysis was that of Gross *(3)*. The total quantity of ethylene thus produced in the seven-day period for this quantity of apples was 0.13 g. It is interesting to compare this with the total quantity of organic material picked up by the 355 g. sample of carbon from the jar containing 6270 g. of apples. This carbon had adsorbed 24 percent of its weight of material, of which 16 percent was accounted for by moisture. Thus 8 percent, or 27.4 g. of organic material had been picked up. At this temperature, therefore, the ethylene accounts for a very small portion (1.7 percent) of the total weight of the evolved material.

In sampling gas atmospheres in commercial storage, special precautions should be observed. Aspiration of the air at a constant rate for determination of ethylene and other volatiles neglects the fact that the ethylene (mass 28) diffuses so much more easily than the other masses, which, as the mass spectra show, go up well above 100. An unbalanced sample may thus be selected unless good air distribution is maintained.

Blank Run on Fresh Carbon. It was thought advisable to check the assumption that all the adsorbed material, except for the normal components of the atmosphere, were due to apple vapor and that fresh activated carbon itself made no contribution. To do this, a 300 ml. (150 g.) sample of carbon was heated to 100° C. (212° F.) and evacuated at high vacuum through three collecting traps cooled in ice, dry ice, and liquid air. Pumping was continued for an hour. The traps were then closed off, and the contents of each admitted to the sample system of the mass spectrometer. After atmospheric and background peaks were accounted for, the result was a complete blank.

Since the volume of the trap was 50 cc., the leak pressure 1.2 cm., and the sensitivity of the instrument 12.5 scale divisions per mm. of gas pressure, and assuming, conservatively, a minimum detectability of 1 scale division and a gas of mass 50, the maximum possible quantity of undetected impurity is 1.3×10^{-8} g. On the basis of a 150-g. sample of carbon, the maximum amount of undetected impurity would be less than 9 millionths of one percent.

INFRARED SPECTRA

As a check on the types of functional groups in apple vapors indicated by the mass spectra, it was thought advisable to examine the infrared spectra of some apple gases.

The use of infrared spectra for qualitative examination of organic compounds depends on the fact that atoms of organic molecules vibrate with definite characteristic frequencies, causing the compound to exhibit selective absorption in the infrared region of the spectrum. In establishing the spectrum, one passes infrared light of all wavelengths through a thin layer of the material being tested. Those wavelengths which correspond to the vibrational frequencies of the molecules are then absorbed; the others are transmitted. Different functional groups, such as aldehyde, alcohol, and ester linkage, show typical infrared absorption frequencies. It is therefore possible to examine the infrared spectrum of a mixture of compounds and get some information on the types of individual compounds therein.

Figure 6 shows the infrared spectrum of the material obtained by adsorption of apple vapors on the 355 g. sample of carbon, followed by vacuum desorption and trapping in ice—salt mixture, and dry ice. The material in these two traps was combined, extracted with one ml. of CCl_4, and used for determination of the spectrum. The spectrum in Figure 6 is a plot of the percent transmitted (I/I_o, where I_o is the intensity through the

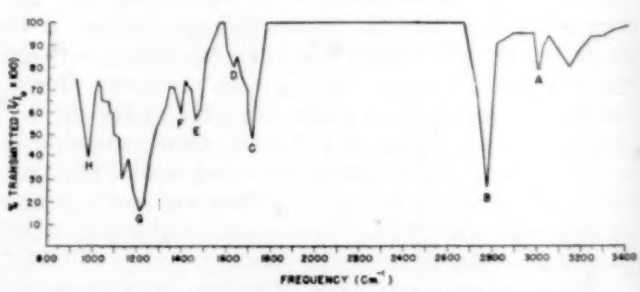

FIG. 6. Infrared spectrum of vapor adsorbed on carbon.

CCl_4 solvent and I is the intensity through the solution being tested, for a cell of thickness 0.1 mm.) as a function of the infrared frequency.

According to compilations of typical infrared absorption frequencies *(1)*, the values shown at (a) (refer to Figure 6) corespond to water or hydroxyl group; (b) is the typical value for C-H stretching, always found in organic compounds; (c) is the frequency of the C = O group in esters or aldehydes; (d) may be exhibited by water, or carbon to carbon unsaturation, such as derivatives of ethylene; (e) is the value for C-H bending, also typical of organic compounds generally; (f) is the value for CH_3, or methyl group; (g) represents the = C — O linkage, typical of esters; (h) usually corresponds to the —CO— linkage, such as may occur in alcohols and ethers. All in all, the spectrum is typical for a mixture of esters, aldehydes and perhaps alcohols, generally confirming the data of the mass spectra.

Figure 7 shows the infrared spectrum of "apple oil," representing the oily organic material obtained by steam desorption (reactivation) of carbon which was used

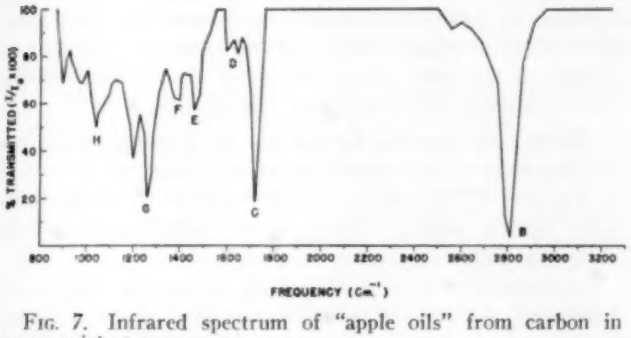

FIG. 7. Infrared spectrum of "apple oils" from carbon in commercial storage.

only for commercial air purification in apple storage warehouses. It is seen that this generally follows the pattern of Figure 6 with the exception that the commercial material does not show absorption in the high frequencies (3000 $cm.^{-1}$) typical of alcohols.

Attempts to obtain material for infrared study by extraction of the carbon with CCl_4 were unsuccessful, showing that the retention of apple gases by carbon is too tenacious for removal even by such a good solvent.

DISCUSSION

The results obtained in this preliminary study show promise for the use of mass spectrometry as a tool in studying apple and other food gases. Some tasks, such as the identification of mixed gases present in very small concentrations in an atmosphere, can apparently be done in no other way now available. Furthermore, the method of direct trapping from the atmosphere into a dry ice or liquid air bath is not subject to any criticism that the nature or proportion of the gases tested is being altered by the method of trapping or testing. Such criticisms might be applied to collection of vapors by steam or flash distillation. Any method of direct sampling from an atmosphere, however, must account for differences in diffusion and condensation.

It should be noted that the storage conditions of the apples in this study did not simulate commercial storage, and therefore no direct translation of the results should be made. It would, however, be extremely interesting to apply these techniques, over a longer period of time, to investigation under commercial storage conditions, since qualitative as well as quantitative changes in "volatiles" production could thus be followed. If it can be verified that the adsorption—desorption technique does not affect the composition of the evolved gases, then a logical further step would involve isolation of a large quantity of this material by desorption from carbon used for commercial air purification in apple storage, separation and identification of the components by techniques such as chromatography, and establishment of mass and infrared spectra of the individual compounds. The use of mass spectrometry in this study would then become much more powerful.

ACKNOWLEDGMENT

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Observations on the Fining of Wines With Casein[a]

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The use of casein for the fining (clarification) of wines was investigated and compared with various other fining agents. Particular attention was given to the details of the fining operations as the amounts of fining agents used, rates of addition, fining temperature, most desirable subsequent storage temperature, etc. The effects of the various fining agents on color and flavor of the wines were also studied.

It is customary to fine and clarify new wines by the addition of such fining agents as gelatin plus tannin, bentonite clay, casein or egg white. In commercial practice these are usually added as dilute aqueous solutions, or in the case of bentonite as a slurry in water or as a powder. The fining agents are precipitated and coagulated chemically as by combination of tannin with gelatin, or by lowering of the pH of the fining solution as of alkaline casein solution by the acidity of the wine or by neutralization of the electrical charge on colloid particles, as of bentonite slurries. The coagulated particles settle out carrying with them the finely divided suspended matter responsible for cloudiness of the wine.

Wine producers are thoroughly familiar with the use of gelatin, tannin, and bentonite in the fining of wines, but the preparation and use of casein finings is much less well understood, in spite of the fact that casein has long been used as a very useful fining agent in European wineries.

Casein of high purity and free of undesirable odor and flavor is now available. It is insoluble in liquids of low pH such as wines and vinegars, but dissolves readily in water at a pH value slightly above neutrality, as at pH 8.0, for example. So-called soluble casein of edible quality is potassium or sodium caseinate. Because it dissolves readily in water without the addition of an alkali, it is very convenient for use as a fining agent. Casein is added to the wine in a slightly alkaline aqueous solution and is precipitated by the acidity of the wine. However, data presented in this paper indicate that some tannin is removed from the wine by the casein and that for satisfactory fining with casein some tannin must be present.

Most treatises on wine making mention the use of casein in fining of wines. For example, Pacottet in 1911 *(10)*, states that casein is often employed in the fining of wines, particularly white wines. He recommends dissolving it in warm water containing two to three grams of $NaHCO_3$ per liter and states that the amount of casein required ranges from 8 to 12 grams per hectoliter of wine. He gives similar information in his French book *(11)*.

Chancrin *(4)* recommends casein fining as a good means of removing excess color from white wines that have become yellow. He advises dissolving 100 grams of casein in 1,000 cc. of 1 percent KOH solution and diluting to 4,000 cc., giving a 2.5 percent solution of casein. Casein as a fining agent has good standing with enologists and is mentioned in many publications *(1, 2, 3, 5, 6, 7, 8, 9, 12, 13, 14, 15)*.

The present investigation has been conducted to establish some of the factors of importance in the use of casein as a fining agent for wines.

EXPERIMENTAL

In winery practice fining agents are added to the wine, not to the unfermented juice. However, if addition before fermentation were to hasten clearing and settling of the newly fermented wine, it would be advantageous. In the present experiments casein in water solution of pH 8 was added to several lots of unfermented, fresh grape juice and the rates of settling and clarity of the new wines observed after fermentation. The treatment did not hasten clarification.

Effect of Casein Content of Fining Solution. In one experiment casein solutions ranging from 0.25 to 5 g. per 100 cc. were used in fining a dry white table wine and a sherry. Two water-soluble and two of water-insoluble caseins were used.

In respect to clarity, the white table wine responded better to fining with solutions of low casein content, 0.25 to 0.50 percent inclusive. With solutions of 1.0 to 5.0 percent the wine was only "clear" to "cloudy" after 48 hours. All of the sherry samples were "very clear" in 48 hours. With the table wine one of the water soluble caseins gave somewhat better clarification than did the other three caseins.

The volumes of the sediments in most of the samples were approximately equal, but tending to be slightly greater with the solutions of low casein content.

Of interest was the relative effect of the various solutions on color retention or removal. The data on this point are shown in Table 1. The values are expressed in terms of percent of color retained, with that of the untreated filtered sample expressed as 100.

Much more color was removed from the dry white wine with solutions of low casein content than by those

TABLE 1

Color Retention in Wines as Affected by Casein Content of Fining Solution

Wine	Casein Sample	Concentration of Casein Solution					
		0.25	0.50	1.0	2.0	3.0	5.0
		Percent Color Retention					
Dry White	1-(soluble)	55	61	87	93	91	91
Dry White	7-(soluble)	56	68	88	96	96	97
Dry White	4-(insoluble)	58	62	100	100	100	100
Dry White	5-(insoluble)	56	57	100	100	100	100
Sherry	1-(soluble)	52	55	59	63	64	66
Sherry	7-(soluble)	54	61	63	68	69	68
Sherry	4-(insoluble)	52	53	56	61	62	63
Sherry	5-(insoluble)	49	49	53	53	53	55

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

of 1 to 5.0 percent. In fact, very little to no color was removed by solutions of 2 to 5 percent casein. The same amount of casein, namely 0.02 g. per 100 cc., was added in all cases, regardless of the casein content of the casein solutions.

Casein content of the fining solution appeared to have little effect on the amount of color removed from the sherry although slightly less was removed by solutions of 1.0 to 5.0 percent casein than by those of 0.25 and 0.5 percent.

Effect of Amount of Casein Added. The amount of casein which will have to be used varies considerably with the type of wine (Claret, Chablis, Riesling, etc.) and with its degree of cloudiness.

In one experiment one water-soluble casein was dissolved in water to give a 2 percent solution and three insoluble caseins were dissolved by adding them to warm water, stirring until well mixed and adding 0.1N NaOH until the caseins dissolved. Enough 0.1N HCl was then added to bring the pH to 8.0 and the solutions diluted to 2 percent casein content.

Three wines, namely a sherry, a dry red table wine, and a dry white table wine, all of the 1947 vintage and about 18 months of age were used. To portions of 100 cc. of the wines were added the four casein solutions. The clarity of the wines, volumes of sediment, and color retention were observed after 48 hours.

The volumes of each of the casein solutions added per 100 cc. were 0.1, 0.2, 0.4, 0.75, 1.0 and 2.0 cc., respectively.

The sherry was slightly cloudy with additions of 0.1 and 0.2 cc. of the soluble casein No. 1, clear with 0.4 and 0.75 cc. and very clear with 1.0 and 2.0 cc. With insoluble casein No. 4 it was very clear with 0.4, 1.0 and 2.0 cc. With insoluble casein No. 2 it was slightly cloudy with 0.1, 0.2 and 0.3 cc., clear with 0.75 cc. and very clear with 1.0 and 2.0 cc. Casein No. 3 gave the poorest results giving a very clear wine only with 2.0 cc. of casein solution. In this experiment 0.02 to 0.04 g. of casein per 100 cc. of wine gave the best results.

The dry red wine was very clear with all additions of all four caseins. In other tests also red table wines responded better than white table wines and sherries to casein clarification.

The dry white table wine responded poorly in this experiment, remaining slightly cloudy in all cases. Later tests showed it to be very low in tannin and that addition of tannic acid greatly improved its clarification with casein.

There appeared to be little to choose between the water-soluble and the water-insoluble caseins in this and in other experiments. Consequently the preference for the soluble casein (caseinate) would seem to be due to its convenience.

In a subsequent experiment with several wines fined with insoluble casein dissolved in 0.03 percent Na_2CO_3 solution and adjusted to pH 8, satisfactory results were obtained on the average at 0.04 g. of casein added per 100 cc. of wine with a settling period of three weeks.

The wines used in these tests were Port, dry white table wine, dry Semillon white table wine, Zinfandel dry red table wine, and a California sherry. The amounts of casein added ranged from 0.01 to 0.06 g. per 100 cc. All except the Port were slightly cloudy after fining with 0.01 and 0.02 g. of casein per 100 cc. but clear with 0.04 and 0.06 g. The Port was cloudy with 0.01 g. per 100 cc., slightly cloudy with 0.02 g. and clear with 0.04 and 0.06 g.

Role of Tannin. In a preliminary experiment a dry white table wine was fined with casein without addition of tannin. To another portion was added 0.1 g. of U.S.P. synthetic tannic acid per 100 cc. and to another portion 0.1 g. of grape seed tannin per 100 cc. To 100 cc. portions of each lot were then added 1 and 2 cc. of a 2 percent casein solution. Both samples of the wine to which no tannin or tannic acid had been added remained cloudy; all samples to which tannin or tannic acid were added became very clear.

Determination of tannin content by the A.O.A.C. method showed a slight but definite decrease in tannin content during casein fining.

In a subsequent experiment four wines, namely, Zinfandel red table wine, an experimental Spanish-type sherry, a 1949 Palomino white table wine and a 1949 orange Muscat white table wine were fined as shown in Table 2. After fining, settling and decanting or after fining and filtering as the case might be, samples were stored one week at 32° F. (0° C.) and others at 120° F. (49° C.) and observations made on clarity in order to determine the effect of each treatment on stability of the wine. The tannin content before and after treatment was determined.

TABLE 2

Effect of Added Tannic Acid on Casein Fining of Palomino Wine

Sample No.	Treatment	Tannic Acid after fining	Nitrogen after fining	Clarity after 1 week at 32° F.	Clarity after 1 week at 120° F.
		mg./cc.	mg./cc.		
1.	No treatment	0.35	0.65	Hazy	Hazy
2.	0.04 percent casein; 3 weeks	0.34	0.59	Clear	Clear
3.	0.04 percent casein, plus 0.04 percent tannic acid; 3 weeks	0.49	0.61	Clear	Clear
4.	0.04 percent casein, filtered immediately	0.29	0.60	Clear	Clear
5.	0.04 percent casein, plus 0.04 percent tannic acid; filtered immediately	0.44	0.69	Hazy and dark	Clear

Readings were taken also on transmission of light of three wave lengths, 520, 580 and 660 mμ by an Evelyn colorimeter for Palomino wine.

Microkjeldahl determinations of nitrogen content were made also to determine whether any casein remained in the wine. The results are shown in Table 2.

Sample number 5 was brilliantly clear after filtering but became hazy at 32° F. (0° C.). Sample number 3 tasted of tannic acid. Evidently some casein remained in sample 5 as its nitrogen content was somewhat above that of the untreated sample. In sample 3 which was treated as was sample 5 except that it was allowed to stand for three weeks and was then drawn off its sediment, there appeared to be no residual casein in solution.

The values given in Table 3 are for percent of light transmitted by the wine in comparison with distilled water as 100 percent, readings being made by Evelyn colorimeter. Casein fining and settling, casein plus tannic acid with three weeks' settling and casein fining with immediate filtration somewhat increased light transmission at the three wave lengths indicating some loss of color representing these wave lengths. Casein plus tannic acid with immediate filtration reduced transmission at the three wave lengths indicating an increase in optical density, i.e. in color.

Storage at 120° F. (49° C.) for one week appeared to increase light transmission somewhat in all samples. At 32° F. (0° C.) there appeared to be little change.

In order to conserve space the data for the other wines will not be tabulated.

With the experimental sherry the results in respect to clarity of samples was similar to those obtained with the Palomino wine, but the increase in light transmission was much greater. Thus the transmission nearly doubled at 520 and 580 mμ on fining in treatments, 2, 3, and 4 and increased nearly 30 percent at 660 mμ. As in other experiments, casein fining removed

TABLE 3

Effect of Casein Fining With and Without Added Tannic Acid on Light Transmission for Palomino Wine [b]

Sample No.	Treatment	Original Wine			After 1 week at 32° F.			After 1 week at 120° F.		
		520 mμ	580 mμ	660 mμ	520 mμ	580 mμ	660 mμ	520 mμ	580 mμ	660 mμ
1.	No treatment	79 [b]	87	83	78	86	82	82	92	87
2.	0.04 percent casein; 3 weeks	84	90	87	85	92	88	86	95	91
3.	0.04 percent casein, plus 0.04 percent tannic acid; 3 weeks	80	88	83	84	92	88	84	93	88
4.	0.04 percent casein; filtered immediately	82	89	86	82	88	86	87	95	91
5.	0.04 percent casein, plus 0.04 percent tannic acid filtered immediately	74	80	76	69	78	74	79	91	85

[b] All values are expressed as percent light transmittance.

Data is given on light transmission at three wave lengths for the treated and untreated wines as determined by the Evelyn Colorimeter.

more color from sherry than from other wines. Treatment 5 increased light transmission somewhat but less than did the other treatments. Also it was the only treated sample to cloud at 32° F.

All of the treated Golden Muscat samples remained clear at 32° F. and 120° F. (0° and 49° C.), and there was no detectable change in the nitrogen content by any of the treatments. Light transmission data were similar to those for the Palomino wine, and similarly for tannic acid content.

Casein removed a measurable amount of tannic acid; slight in some cases, but appreciable in others. For example, the tannin or tannic acid content of the untreated Golden Muscat wine was 0.35 mg. per cc. and of that fined with casein was 0.30 mg. per cc. That fined with casein plus tannic acid contained 0.47 mg. of tannic acid per cc.; if none had been removed, it should have been 0.75 mg. per cc. The amount of tannin or tannic acid removed was less than the weight of the casein added in all cases.

With the Zinfandel red table wine, there was only slight to no loss in color. Tannic acid removal by the casein was somewhat larger than in the white wines. Thus 0.04 percent casein alone removed 0.25 mg. tannic acid per cc. or 0.025 per 100 cc.; with 0.04 percent casein plus 0.04 percent added tannic acid, the decrease was 0.30 mg. or 0.030 g. per 100 cc. Thus 4 g. of casein removed from 2.5 to 3.0 g. of tannic acid. The treated Zinfandel samples were all stable at 32° F. and 120° F.

Relation of Formol Titration to Color Removal. The formol titration values of two soluble caseins designated as A and B and of three insoluble caseins, C, D and E were determined by neutralizing a 2 percent solution of casein with 0.1 N NaOH to phenolphthalein and adding 10 cc. of neutralized 40 percent formaldehyde. The sample was again neutralized, and the number of cc. of 0.1 N NaOH required for the second titration was expressed as the formal titration values.

Three wines were then fined with the five caseins in the usual manner and the effect of the various caseins observed on color removal, that is, on light transmittance at 520 mμ for the two sherries and at 660 mμ for the red Zinfandel. The formol titration values and light transmittances are given in Table 4.

TABLE 4

Relation of Formol Titration Values of Several Caseins and their Effect on Light Transmittance

Casein Sample	Formol Titration Value	Experimental Spanish Type Sherry Transmittance at 520 mμ	California Sherry Transmittance at 520 mμ	Zinfandel Red Wine Transmittance at 660 mμ
A	44.50	71	72	45
B	41.15	64	67	40
C	38.81	60	62	36
D	36.73	57	59	34
E	35.10	54	55	33
		Control: 29	Control: 20	Control: 32

There appears to have been a definite relationship between the formol titration and the decolorizing power of the five caseins; the higher the formol titration the greater was the decolorizing power, or the greater the light transmittance. Therefore, if the wine maker should wish to clarify a wine without much loss of color, he would choose a casein of low formol titration value; if, on the other hand, he should wish to remove excess color from a sherry or white table wine, he would choose a casein of high formol titration value.

Comparison of Casein, Bentonite and Gelatin as Fining Agents. In this experiment a dry white table wine and a sherry were fined with eight different amounts of 2 percent casein, gelatin, and tannin solutions. The additions ranged from 0.2 cc. to 4.0 cc. per 100 cc. of wine. The tannin was added before the gelatin. No tannin was used with the casein. The amounts of bentonite used ranged from 0.6 cc. to 4 cc. of a 5 percent slurry per 100 cc. of wine.

All samples fined with bentonite became brilliantly clear within 48 hours. The dry white wine became very clear but not brilliant at all additions of the casein.

The dry red wine became very clear with additions of 0.2 to 0.8 cc. of 2 percent casein solution and brilliant with additions of 1.0 to 4.0 cc. The sherry behaved similarly to the dry red wine.

With 0.2 cc. of tannin and gelatin solutions the dry white remained cloudy; it became clear with 0.4 and 0.6 cc. and very clear with 0.8 and 2.0 cc. of the solutions. The dry red wine was very clear with all additions of these finings. The sherry was cloudy with 0.2 cc., clear with 0.4 and 0.6 cc., and very clear with 0.8 and 1.0 cc. and again cloudy with 2.0 cc. of the solutions.

The volumes of the bentonite sediments were much larger than those of the casein and the gelatin plus tannin. The bentonite and gelatin-tannin sediments were fine grained and the casein coarse.

Table 5 shows the data on color retention in comparison with that of the untreated wine.

TABLE 5

Color Retained After Fining with Casein, Bentonite and Gelatin Plus Tannin [c]

Wine	Fining Agent	Amount of Fining Agent Used Per 100 cc. Wine							
		0.2 cc.	0.4 cc.	0.6 cc.	0.8 cc.	1.0 cc.	2.0 cc.	3.0 cc.	4.0 cc.
		Percent Color Retained							
Dry White	Casein	95	89	87	80	74	69	59	55
Dry White	Bentonite	....	....	97	....	92	86	82	82
Dry White	Gelatin-Tannin	123	92	86	77	74	70	....	....
Dry Red	Casein	100	100	100	100	88	86	86	80
Dry Red	Bentonite	....	....	90	....	89	76	71	60
Dry Red	Gelatin-Tannin	88	92	92	92	94	88	....	....
Sherry	Casein	80	77	67	61	57	55	49	45
Sherry	Bentonite	....	....	80	....	78	77	73	73
Sherry	Gelatin-Tannin	88	80	65	63	62	....	....	....

[c] The fining solutions contained 2 percent of casein, tannin or gelatin or 5 percent Bentonite.

An unexpected finding was that gelatin plus tannin fining had nearly as much decolorizing action as did casein and that bentonite also exerted considerable decolorizing action. In the dry white wine the added tannin increased the color materially.

In general the bentonite gave clearer wines than did casein or gelatin plus tannin finings, and casein was somewhat superior to the gelatin plus tannin.

The fined wines were racked (drawn off) and stored at 37° F. (2.8° C.), 120° F. (49° C.), and 0° F. (—18° C.) several days and then brought to room temperature for several days. At 37° F. the wines fined with gelatin plus tannin became cloudy; those fined with bentonite or casein remained clear to brilliant with no development of sediment. At 120° F. the dry white wine fined with casein and with gelatin-tannin became slightly cloudy; all of the dry red and sherry samples remained clear.

At 0° F. (—18° C.) all samples including the controls remained very clear; in fact, they improved in clarity on thawing. Evidently freezing exerted a clarifying action. All samples showed considerable sediment after thawing; much of which was cream of tartar.

It appeared that in these experiments that bentonite fining gave greater stability than the other two finings and that casein was superior to gelatin plus tannin in this respect.

In later experiments four wines fined with casein, and with casein plus tannic acid proved to be stable at 32° F. and 120° F. except when fined with casein plus tannin and filtered at once. In this case clouding occurred with two of the wines, a sherry and a dry white, at 32° F. The dry white wine fined with casein and tannic acid and filtered at once, darkened considerably several weeks after removal from 120° F. storage.

Effect of Temperature of Fining. In commercial practice wines have been heated during fining with bentonite, but insofar as we know heating has not been practiced with other fining agents. Four wines (a dry red, dry white, sherry and Port) were fined with 1 and 2 cc. of 2 percent casein solution per 100 cc. of wine at (a) room temperature, (b) 120° F. (49° C.), (c) 140° F. (60° C.), and (d) 160° F. (71° C.); and allowed to settle for 48 hours.

At room temperature all four wines were very clear with both amounts of fining agents; at 120° F. the dry wines were slightly cloudy and the two fortified were clear. At 140° F. and 160° F. all samples of all four wines were cloudy.

Subsequent experiments confirmed these observations. Therefore, it appears that casein fining should be done at cellar temperature.

In several experiments wines were fined with 2 percent casein solution and after standing a few minutes 5 percent bentonite solution was added. Settling of the finings was rapid, and the wines usually became very clear to brilliant.

EFFECT OF CASEIN AND BENTONITE ON IRON AND COPPER CONTENT OF WINE

The presence of small amounts of copper and iron dissolved from winery equipment often causes clouding of white wines. At present the only effective method of removing these traces of metal is by treatment with potassium ferrocyanide, to which some objection has in the past been made by regulatory agencies.

In some experiments 2.4 to 5.0 p.p.m. of copper, in the form of a soluble salt, were added to dry white wine and to sherry and the wines were fined with various amounts of casein, bentonite, and casein plus bentonite. Only small proportions of the copper were removed. Similar results were obtained in fining the same wines to which 10 to 11 p.p.m. of iron as a ferrous salt were added. Therefore, under the conditions of our experiments casein and bentonite had very little value in reducing copper and iron contents of wines.

Effect of Casein Fining on Flavor. In one series of tests, five aged wines (Sauvignon blanc dry white, Semillon dry white, Burgundy, Sherry and Muscatel sweet fortified) were fined with casein, racked, bottled and compared by several experienced tasters in respect to flavor and bouquet with the untreated wines. No difference or very little difference in flavor and bouquet could be detected in the Sauvignon, Semillon, Burgundy and Muscatel. Several tasters thought the flavor and bouquet of the sherry had been modified noticeably by casein fining; but as its color had also been reduced materially, this change may have influenced the tasters' judgment. Several tasters (including two of the authors) thought the flavor of the sherry had become less harsh and thus improved by the casein fining.

In a more recent experiment three brilliantly clear white wines (two Semillons and a Riesling) were fined with casein, and then tasted. The clear wines were used to avoid giving to the tasters any knowledge of the treatment the wines had received. Insofar as experienced tasters could determine, the casein fining did not damage the flavor or bouquet of two of the wines but reduced the bouquet of one somewhat.

CASEIN FINING IN CONJUNCTION WITH PECTINOL

Some wineries add a pectic enzyme, usually Pectinol-O, to the juice before fermentation or to the new wine after fermentation. It hydrolyses pectic substances and thereby greatly hastens settling and clearing of the wine after fermentation.

In order to ascertain whether added pectic enzyme and casein fining were compatible, samples of a new Semillon wine were treated as follows: Sample (a) was untreated control, (b) was treated with 2 cc. of a 2 percent casein solution per 100 cc., (c) was clarified with 0.3 g. of Pectinol-O per 1,000 cc. and (d) obtained both casein and Pectinol treatment. After standing for several weeks (a) was unchanged, (b) and (c) were clear and (d) was slightly cloudy. Further experiments are necessary before any definite conclusions can be reached although the indication in this experiment is that casein and Pectinol should not be added simultaneously.

COMPARISON OF CARBON AND CASEIN FOR REMOVING EXCESS COLOR FROM DARK SHERRY

Frequently sherry becomes too dark in color during aging. The usual method of reducing the color is by treatment with decolorizing carbon under special permission of the local federal agent.

In several experiments sherries were treated with the usual amounts of decolorizing carbon from a winery and other samples with casein.

The samples fined with casein retained the flavor and bouquet of the original wines; those treated with the carbon developed a distinct off flavor (so called "carbon flavor") and lost much of the original bouquet.

Both treatments were effective in removing excess color, giving sherries of pale dry color. But in our experiments casein fining yielded superior wines.

COMPARISON OF SEVERAL METHODS OF ADDING CASEIN FININGS

To dry Semillon white wine 2 percent casein solution was added at the rate of 2 cc. per 100 cc. in the following manner: To sample (a) the casein was added rapidly in a single "dose," followed by mixing, to sample (b) the casein was added in very small fractions with constant mixing, and to sample (c) it was added to 10 percent of the wine and mixed, and then added to remaining 90 percent of the wine.

Wine (c) settled more rapidly than (a) and (b); but after 24 hours all three lots were very clear and had about the same volumes of sediment.

SUMMARY

1. The use of casein in the fining (clarification) of wines is an old and well established winery practice.

2. Addition of casein to grape juice before fermentation did not noticeably improve clearing of the new wine after fermentation.

3. In dry white wine casein solution of low concentration gave better results than those of high concentration.

4. Casein solutions of low casein content (0.25-1.0 percent) removed more color from dry white wine than solutions of higher concentration (1 to 5 percent).

5. The quantity of casein required to fine wines varied with their type and with the degree of cloudiness, but in most cases 0.04 to 0.06 grams of casein per 100 cc. of wine gave good results.

6. Addition of U.S.P. tannic acid or of grape tannin greatly improved clarification by casein of wines deficient in tannin. The tannin content of wines was reduced by casein fining although the amount of tannin removed varied markedly and in most cases was much less than the amount of casein added.

7. In red wines very little color was removed by casein fining, in sherry considerable to marked reduction in color occurred at higher additions and in dry white wine moderate to considerable reduction in color occurred. However, and this is very important from the standpoint of the federal wine regulations, as great to nearly as great color reduction (color removal) occurred with gelatin fining as with casein fining of sherry and dry white wines.

8. There appears to be a definite relationship between the formol titration value of a casein and its decolorizing power. The higher the formol titration of the casein the greater was its decolorizing power.

9. In most cases fining with bentonite gave clearer wines than fining with gelatitn or casein but gave much larger volumes of sediment.

10. Allowing a wine fined with casein to settle followed by racking (drawing off), gave a more stable wine than filtration of the wine immediately after adding casein fining.

11. Stability of fined wines as judged by clouding upon storage at 120° F. and 37° F. was best with bentonite fining but better with casein fining than with gelatin fining.

12. Fining of wine with casein at room temperature gave much better results than at the elevated temperatures tested, namely 120° to 160° F. (49° to 71° C.).

13. Fining of wine with casein plus bentonite gave rapid settling of the finings and brilliant clarity in most cases. Perhaps in commercial practice this combination would prove very useful.

14. Casein fining removed practically none of the copper and iron from wines to which small amounts of salts of these metals had been added.

15. Use of a commercial pectic enzyme in conjunction with casein fining appeared not to enhance the effect of the casein, but further experiments on this point are necessary.

16. The flavor of sherry of which the color was reduced by treatment with decolorizing carbon was poorer than that treated with casein to about the same color.

17. Before fining a commercial size lot of wine with casein or any other fining agent, small samples should be fined experimentally to determine whether the wine will respond satisfactorily and what amount of fining agent is required.

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The Use of Antioxidants in Deep Fat Frying[a]

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The addition of an antioxidant after the cooking process has proven successful on a commercial scale in increasing the storage life of fried potato chips, nuts, pork skins, and other deep fat fried food products. An antioxidant-salt product added to a hot fried food immediately after frying effectively retards rancidity development. With certain types of nuts the antioxidant is added to the dressing oil in which the cooked nuts are immersed for glazing purposes.

Heating of oils at the cooking temperatures of 350°-425° F. (176.5-218° C.) normally used in deep fat frying rapidly results in the oxidation or destruction of NDGA, propyl gallate and butylated hydroxyanisole. There appears to be no relation between the carry-through of an antioxidant in baked goods and its resistance to oxidation when cooked in oils at a high temperature.

Until recently the food industry was dependent on naturally occurring antioxidants such as lecithin for the stabilization of edible fats and oils, but the natural antioxidants were not sufficiently effective to prevent large economic losses resulting from rancidity development. Highly active antioxidants such as hydroquinone were found too toxic for use in food products. The development of nordihydroguairetic acid (NDGA *(1)* and the esters of gallic acid *(13)* of low toxicity *(12)* led to their approval by the Food and Drug Administration of the Federal Security Agency. NDGA and propyl gallate when used in combination with synergists showed their powerful stabilizing action on fats, and their use increased rapidly until, at the present time, roughly 90 percent of the lard industry uses one or the other of these two antioxidants for the protection of lard. More recently, butylated hydroxyanisole was included as an approved antioxidant *(7)*, and, although this product is a relatively weak antioxidant for increasing shelf-life, it has the property of carry-through into baked goods, and has therefore found wide usage in the stabilization of animal fats, particularly lard.

The first large commercial use of these antioxidants was in their application to lard. NDGA, which is relatively insoluble in lard, was dissolved in an alcoholic solution in combination with citric acid and added to the lard which was then heated to remove the alcohol. Propyl gallate was formulated *(1, 2)* into an oil-soluble combination sold under the trade-name of G-4 [b] whose composition included propyl gallate, citric acid, lecithin and corn oil. G-4 is readily soluble in oils and melted fats, and the manufacturers recommend that 8 ounces of G-4 be added to 1000 pounds of lard in the final manufacturing step. Butylated hydroxyanisole is now sold in combination with propyl gallate and citric acid in propylene glycol under the trade-names of Tenox II [c] and Sustane [d].

[a] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 23, 1950.

[b] Manufactured by Griffith Laboratories, Chicago, Illinois.

[c] Manufactured by Tennessee Eastman Corp., Kingsport, Tennessee.

[d] Manufactured by Universal Oil Products Co., Chicago, Illinois.

ANTIOXIDANTS FOR DEEP FAT FRYING

The application of antioxidants to the deep fat frying industry has involved many problems. The oils are cooked at temperatures ranging from 350 to 425° F. (176.5 to 218° C.) and under these conditions the fats oxidize and lose stability. The problem of quality control and rancidity prevention has been of paramount importance to the potato chip industry and to the deep fat friers of nuts and other products. In order to avoid rancidity, certain manufacturers have found it advisable to use only hydrogenated shortenings in their cookers. However, other manufacturers are convinced that a better flavor is obtained by using vegetable oils.

Considerable work has been done to determine the most efficient methods to improve the shelf life of deep fat fried products by the use of antioxidants. Initial investigations were made by adding the antioxidants to the cooking oil under laboratory conditions, and it was found that this increased the shelf life of potato chips and nuts. Potato chips were fried in oils with and without 0.05 percent G4, and the antioxidant-treated chips showed twice as long shelf life.

Commercial scale tests were then made at three large potato-chip companies, and each test extended for one week of operation in which samples of the chips and the cooking oils were taken at 4-hour intervals. One cooker was used for the untreated oils while the second cooker contained oil with 0.05 percent G-4, and the same amount of antioxidant was added to the feeding tank. The cooking oils and the chips were then checked for stability, and it was found that in the plant the potato chips taken from the cooker containing antioxidant within two hours of commercial operation showed increased shelf life over the controls, but as the test continued this beneficial effect was rapidly lost. Other tests in which higher concentrations of antioxidants were used resulted in substantially similar negative results.

Studies were made then to investigate the decomposition rate of propyl gallate at temperatures of 325 to 375° F. (162 to 190° C.) in which the heating tests were made using lard, corn oil, and cottonseed oil. The cooking tests were made using one pint of oil or fat contained in one-quart stainless steel beakers partially immersed in a thermostatically controlled oil bath . The effect of length of cooking at 325° F. (162° C.) and 375° F. (190° C.) on the retention of propyl gallate in lard is given in Table 1.

TABLE 1

Effect of Temperature and Time of Heating on Concentration of Propyl Gallate

Time of Heating, Hours	Percentage of Propyl Gallate in Lard	
	325° F. (162° C.)	375° F. (190° C.)
0	0.0058	0.0058
1	0.0052	0.0048
2	0.0050	0.0037
8	0.0032	0.0008
16	0.0002	0.0000

The propyl gallate concentration was determined *(9)*, and it was found that heating at 325° F. (162° C.) for 8 hours reduced the propyl gallate concentration from 0.0058 percent to 0.0032 percent. The temperature of 375° F. (190° C.) more closely approximates the commercial temperatures used and, at this temperature, the decomposition occurs more rapidly, and substantially all the antioxidant is lost within 8 hours. Tests with corn and cottonseed oils yielded substantially similar results. These data afforded an explanation for the discrepancy found between laboratory tests in which the oils are heated for only a short interval and large scale commercial tests where heating is continued until all the oil is consumed or unfit for use.

TENOX II VERSUS G-4 IN DEEP FAT FRYING

It has recently been reported *(10)* that Tenox II has unique properties, being able to carry-through the heating process in deep fat frying, whereas both NDGA and propyl gallate lack this property of carry-through. Studies were made to check on the relative value of Tenox II as compared to G-4 in deep fat frying.

Tenox II and G-4 respectively were added to lard on the basis of 0.05 percent, and the lards were then heated to 375° F. (162° C.). Samples of the lard were taken at intervals and checked for stability, ordinarily expressed in terms of hours to become rancid while being tested by the active oxygen method (A.O.M.) *(6)*. The results are given in Table 2 and show that the

TABLE 2

Effect of Heating Upon Stability of Lard Containing Tenox II and G-4

Time of Heating at 375° F. (190° C.), Hours	Stability of Lard, A.O.M., Hours		
	Control	0.05 percent Tenox II	0.05 percent G-4
0	6	57	48
4	0.5	14	13
8	0.5	3	3

stability of lards containing either Tenox II or G-4 decreases very rapidly during the heating process.

The original lard containing 0.05 percent Tenox II had an A.O.M. value of 57 hours, and after 4 hours of cooking at 375° F. (190° C.) this value was reduced to 14 hours, being equivalent to the value obtained with G-4. Cooking for 8 hours reduced the A.O.M. value to less than the original control lard for both antioxidants.

In a more practical test of carry-through during heating, the conditions used for obtaining the data given in Table 2 were repeated except that potato chips were fried in the lard at hourly intervals and the fried potato chips were then incubated at 140° F. (60° C.) in loosely capped 4-ounce jars, using the Schaal Method *(5)*. The results given in Table 3 show that Tenox II and G-4 both yield potato chips with greatly decreased shelf life

TABLE 3

Stability of Potato Chips Cooked in Lard Containing Tenox II and G-4

Hours of Heating Lard at 375° F. (190° C.) before Potato Chips Were Fried	Stability of Potato Chips Incubated at 140° F. (60° C.), Hours		
	Control	Lard Plus 0.05 percent Tenox II	Lard Plus 0.05 percent G-4
0.5	20	220	110
3	12	34	18
6	4	12	12
9	....	6	6

after continued heating of the lard. These results as well as others do not indicate that Tenox II has unique properties as compared to G-4 or NDGA with respect to its resistance to decomposition during high temperature cooking.

An additional test was made using Tenox II. A 50-50 mixture of hydrogenated fat and lard containing Tenox II was used for deep fat frying tests. The fat was weighed into two Pyrex containers and 0.10 percent Tenox II was added to one sample while the other was kept as the control. The fats were heated to 375° F. (190° C.) and potato chips were fried in the heated oils at hourly intervals. The stability of the potato chips was checked by incubation at 140° F. (60° C.). The results obtained are given in Table 4.

TABLE 4

Use of Tenox II In Deep Fat Frying of a Mixture of Lard and Hydrogenated Fat

Hours of Heating Fat Mixture at 375° F. (190° C.) Before Potato Chips Were Fried	Stability of Potato Chips Inculated at 140° F. (60° C.), Days	
	Control	0.10 percent Tenox II
0.5	30	34
2	7	12
4	3	6
8	3	6

The Tenox II shows the same behavior in a lard-hydrogenated fat mixture as it does in lard alone. A rapid breakdown of the Tenox II occurs during the cooking process, and within a few hours the effectiveness of the antioxidant is largely lost.

On the basis of commercial as well as laboratory tests it has been concluded that the known acceptable antioxidants cannot be successfully applied to the deep-fat frying industry by adding the antioxidant to the oils in the cooker. The antioxidants are rapidly oxidized, and the beneficial effect of their addition does not effectively carry-through to the fried potato chips, nuts, or other products.

CARRY-THROUGH OF ANTIOXIDANTS

The term carry-through in an antioxidant was originally used with respect to the baking industry. In general, lard containing BHA yields pie crusts and crackers of greater shelf life than lard containing propyl gallate or NDGA, and the term carry-through was used to denote this difference. Recently *(10)* the term carry-through has also been applied to the deep fat frying industry with the implication that the same factors apply to both baking and deep fat frying. Carry-through in baked goods is the result of factors of which little is known. Sufficient information is now available, however, to show that there is no relation between carry-through in baked goods and the term carry-through in deep fat frying. According to Mattil and Black *(10)*, carry-through in baked goods can be explained on the basis of the solubility in fat of the antioxidant.

Studies were made to check on this hypothesis of carry-through. A series of antioxidants of varying fat solubility was added to a lard on the basis of 0.01 percent plus the addition of 0.003 percent citric acid. The method of Mattil & Black *(10)* was then used to determine the percentage retention of the antioxidant in the fat layers, after the washing with hot water. The stabili-

ties of the original lards were determined, and these same lards were then baked into pie crusts, using 120 grams of flour, 50 grams of lard, ½ teaspoon of salt, and 2 tablespoons of water. The stability of the pie crusts was then determined at 140° F. (60° C.). The relationship between fat solubility, antioxidant value, and pie crust stability is shown in Table 5.

TABLE 5

Relation Between Fat Solubility and Carry-Through of Antioxidants

Antioxidant	Percentage Retention of Antioxidant in Fat Layer after Water Wash	A. O. M. of Lard, Hrs.	Storage Life of Pie Crusts at 140° F. (60° C.), Days
Control	---	6	2-3
Hydroquinone	5	90	2-3
Propyl Gallate	36	70	3-4
NDGA	38	78	3-4
Hexyl Gallate	61	40	4
Butylated Hydroxy Anisole	72	32	16
Lauryl Gallate	74	36	4-5
4-Lauryl Catechol	75	53	7-8

As shown by the results given in Table 5, the percent retention of the antioxidants in the lard varied from 5 to 75 percent, while the stability of pie crusts varied from 2-3 to 16 days. The results do show some slight relationship between fat solubility and carry-through, but this effect is not great. It will be noted that three antioxidants, namely BHA, lauryl gallate and 4-lauryl catechol had very comparable fat solubilities, and yet BHA pie crusts show 3-4 times the stability of lauryl gallate pie crusts and 2 times the stability of the pie crusts baked using 4-lauryl catechol. It is therefore indicated that carry-through cannot be properly explained on the basis of the fat solubility of an antioxidant.

The results given in Table 5 show the profound pro-oxidant effect on the baking process. The lards stabilized with hydroquinone, propyl gallate or NDGA yield A.O. M. values of 70 to 90 hours, which means that air can be passed through these lards heated to 212° F. (100° C.) for 3 days before they go rancid. These same lards when mixed with flour, a little salt, and water and then baked in the oven for only 10 minutes at 450° F. (232° C.) yield pie crusts which go rancid on storage in closed bottles within 2 to 4 days at a storage temperature of 140° F. (60° C.). The same doughs without the baking process remain stable at 140° F. (60° C.) for weeks. Some unusual reaction occurs during this 10-minute baking process which destroys the effectiveness of these antioxidants.

In studies made with BHA, instances have been found when this antioxidant will not carry-through the baking process. Tests have been made with various commercial lards, and one of the lards tested showed a good response to BHA in terms of A.O.M. stability, but for some undetermined reason BHA would not carry-through the baking process when this lard was used. The results obtained for three different lards using varying concentrations of BHA along with 0.003 percent of citric acid are given in Table 6.

The carry-through with BHA as shown in Table 6 progressively increased in two of the lards with increasing concentration from 0.003 to 0.015 percent, whereas the third lard with an initial A.O.M. value of 1.5 hours exhibited no carry-through at even the highest

TABLE 6

Carry-Through of BHA at Various Concentrations in Different Commercial Lards

Concentration of BHA, percent	Stability of Pie Crusts at 140° F. (60° C.), Days		
	Good Quality Steam Rendered Lard. A. O. M. Hrs. : 4	Kettle-Rendered Lard	
		A. O. M. Hrs. : 1.5 No. 1	A. O. M. Hrs. : 1.5 No. 2
Nil	1	1	1
0.003	3-4	2	1
0.005	5-6	4	2
0.0075	5-6	4	---
0.01	8	6	1-2
0.015	14	7	1-2

concentration of BHA used, even though the use of 0.01 percent BHA did increase the A.O.M. value of this lard from 1.5 to 22 hours. Other samples of lard from this same manufacturer yielded similar results. Some ingredient in this lard was destroying the effectiveness of B.H.A. These data are presented as a caution to show that there are many factors in carry-through which can result in variable results.

Another factor which plays a role in carry-through is the age of the lard. A freshly prepared lard gives a better carry-through in baked goods than a lard stored for four months at room temperature before baking. If an antioxidant such as G-4 is added to the lard during manufacture, however, the carry-through remains unchanged for a six-month storage period and longer. For example, after four months of storage the control lard yielded pie crusts with a storage life of 2 days at 140° F. (60° C.) whereas the lard containing G-4 yielded pie crusts with a storage life of 7 to 8 days, a value similar to that obtained for the fresh lard.

It is indicated that the most important factor contributing to carry-through is the flour used for the baking tests. The stability of pie crusts made with a single control lard has varied from 1 to 8 days when different flours were used. These flours included cracker and bleached flours from different manufacturers. With this same series of flours the pie crusts made with BHA have varied in stability from 12 to as long as 35 days.

Some factor in the flour plays an important role in carry-through. Inasmuch as flours contain traces of metals, including iron and copper which are pro-oxidants, it was thought that this variation in carry-through might be attributable to variations in metal content. However, the addition of metallic de-activators did not result in any increase in shelf life, casting doubt on the importance of this possible factor.

A further experiment more clearly demonstrates the important role of flour in carry-through. A sample of cracker flour was heat-moisture treated by the method used by Sair and Fetzer *(14)* in their studies on starches. In this procedure the starch or flour is subjected to steam at 5 pounds pressure for 30 minutes. This heat-moisture treatment results in no apparent microscopic or visible change in the flour. However, a profound physical change does occur in which the starch and proteins are retrograded or denatured. If the heat-moisture treated flour is then used for pie crusts it has a marked pro-oxidant effect, and even the pie crusts containing BHA go rancid, as rapidly as the control, as

TABLE 7

Carry-Through of BHA Using Heat-Moisture Treated Flour

Flour	Shelf Life of Pie Crusts at 140° F. (60° C.), Days		
	Control Lard	Lard with 0.05 percent Tenox II	Lard with 0.01 percent Hydroquinone plus 0.003 percent Citric Acid
Commercial Cracker Flour	4	18	5
Heat-Moisture Treated Commercial Cracker Flour	2	2	2

shown by the results given in Table 7. These data yield further confirmation that carry-through in baked goods is tied up with factors in the lard and in the flour.

APPLICATION OF ANTIOXIDANTS TO POTATO CHIPS AND FRIED NUTS

As pointed out previously, according to our present knowledge, the successful application of antioxidants to deep fat frying cannot be made by adding the antioxidant to the cooker. The antioxidant is rapidly oxidized and its addition results in little improvement of either the oil itself or the products cooked therein. The best solution at present for the protection of deep fat fried products is the use of an antioxidant salt or dressing oil which permits the complete utilization of the antioxidant *(3, 4)*. The successful application of antioxidants to the deep fat frying industry is obtained by adding the antioxident after the potato chips or nuts are fried. Upon completion of the frying process, the hot fried potato chips are commercially conveyed under a hopper which salts the chips on the basis of 1 to 3 percent.

Typical commercial results obtained by the use of antioxidant salt in the commercial production of potato chips are shown in Table 8.

TABLE 8

Effect of Using Antioxidant Salt Upon the Stability of Potato Chips

Company No.	Storage Life of Potato Chips at 140° F. (60° C.), Days	
	Regular Salt	Antioxidant Salt
1	1	2-3
2*	45*	65-70
3	5	10-12
4	8	14-16
5*	35*	45
6	7	9-10

* Hydrogenated Vegetable Oils.

The antioxidant salt definitely increases the shelf life of potato chips, and, as indicated by the above results, this advantageous effect is obtained with both hydrogenated and unhydrogenated vegetable oils. The antioxidant salt used in these tests has now been sold commercially for two years. The above results are typical of a large number obtained during this period, and they indicate that the shelf-life of the potato chips can be increased by 50 to 200 percent by the use of an antioxidant salt.

Many commercial tests have also been made in the nut industry. Fried nuts are usually salted batch-wise, while in other industrial plants the fried nuts are immersed in a vegetable dressing oil to enhance their appearance. The application of antioxidants to the fried nut industry has been accomplished successfully by the use of an antioxidant salt or an antioxidant dressing oil.

In order to enhance the flavor, certain commercial plants add butter to the hot fried nuts. The nuts are fried at 375 to 450° F. (190 to 232° C.) in hydrogenated oil. After the frying process, butter and salt are added to 100 pounds of fried nuts on the basis of 1 pound and 2.5 pounds, respectively. The comparative stabilities of the nuts salted with regular and antioxidant salt are shown by the results given in Table 9.

The antioxidant salt markedly improved the storage life of the nuts coated with butter after the frying operation, giving in the case of Red Coats and pecans an increased shelf life of 4 to 500 percent.

A second manufacturer does not use the butter coating treatment after frying, and at this plant the results obtained are given in Table 10.

TABLE 9

Effect of Using An Antioxidant Salt for Stabilizing Deep Fat Fried Nuts[f]

Nuts	Storage Life of Nuts at 140° F. (60° C.), Days	
	Regular Salt	Antioxidant Salt
Red Coats	3-4	17-20
Pecans	9	35
Cashews	off odor after 28 days	good after 28 days

[f] Nuts coated with butter after frying.

TABLE 10

Effect of Using An Antioxidant Salt for Stabilizing Fat Fried Nuts[g]

Nuts	Storage Life of Nuts at 140° F. (60° C.), Days	
	Regular Salt	Antioxidant Salt
Red Coats	5	14-16
Spanish Nuts	8-9	13
Pecans	off odor in 30 days	good after 50 days
Duchille	off odor in 30 days	good after 50 days

[g] No butter applied to nuts after frying.

In the absence of butter the relative advantage of using antioxidant salt is decreased, but especially in the case of Red Coats a substantial improvement in shelf life is obtained.

Results comparable to the above have been obtained by using an antioxidant dressing oil for nuts rather than an antioxidant salt. G-4 is added to the dressing oil on the basis of 16 ounces per 100 pounds, and the immersion of the fried nuts in this solution materially extends the shelf life of the nuts.

SUMMARY

Temperatures normally used in commercial deep fat frying operations rapidly decompose or volatilize the antioxidants added to the cookers. In order to increase shelf life of deep fat fried products, it has been found necessary to add the antioxidants after the frying operation. In the case of potato chips, the antioxidant is mixed with the salt and is then incorporated on the chips during the salting process. This same procedure can be used for deep fat fried nuts, or an alternative procedure for nuts is to add the antioxidant to the "dressing oil" often used to give the fried nuts a more desirable appearance. This same procedure of incorporating the antioxidant salt can be used for all deep fat fried products.

The factors underlying the carry-through of an antioxidant in baked goods are dependent on the antioxi-

dant, the fat, and the flour. There appears to be no relation between carry-through in baked goods and the ability of an antioxidant to resist decomposition during the deep fat frying process.

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Methods of Measuring the Effect of Processing on the Nutritive Value of Fish Proteins[a]

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A REVIEW

The effect of processing on the nutritive value of proteins is reviewed on the basis of nitrogen distribution, quality indexes, chemical scores, efficiency ratios (enzyme activity), and amino acid content. It is generally agreed that amino acid composition does not reflect changes in nutritive value effected by heat, storage, dehydration, unless some measure of decomposition has taken place. Rate of liberation of essential amino acids on in vitro hydrolysis has given sensitive and reproducible tests of protein changes. The need of measuring nutrients as they are modified by processing is discussed.

Papers entitled "nutritive value . . . " have been published on a great many organic substances used for human and animal diets. Results are usually expressed as protein quality index *(1)*, chemical score *(2)*, digestion index *(6)*, biological value *(2)*, protein efficiency ratio *(2)*, apparent digestibility *(12)*. They represent chemical and in vitro tests on food products, chemical, morphological, growth, maintenance, production studies on experimental animals.

The purpose of this paper is to review briefly laboratory measures of nutritive value. To properly process, store, and utilize fishery products, the fisheries technologist must have accurate, reproducible, sensitive tests, applicable to laboratory control of plant production. They must not only detect changes in nutritive value but also provide an insight into the reasons for these changes. The time may come when the nutritive value of canned and frozen fish has as much economic importance to the processor as the mechanics of processing. Fish meals are ready now to make the transition from the status of reduction products to chemical compounds. Yet before the market reflects exact values of nutrients, we must be able to express them as they are modified by mechanical and chemical treatments.

We know that chemical analysis of nitrogen does not measure a difference in quality *(7)*. Only in chronic inanition is there a measurable difference (decrease) in total nitrogen or non-protein nitrogen of experimental animals *(15)*. Nitrogen distribution has been suggested as an index of nutritive value of fishery products *(8)*. Straight chemical tests of nitrogen fractions are correlated more with spoilage than with nutritive value. Lassen *(8)*, studying storage of fish press liquor before condensation to solubles, found that as true-protein nitrogen decreased, non-protein and ammonical nitrogen increased. The growth-promoting ability of the product and also the amino acid content showed corresponding decreases with loss of true protein nitrogen. Total amino nitrogen by formol titration increased with decrease of true protein and decrease in amino acid content.

For years we have been unable to arrive at an objective test for spoilage on this basis, due largely to different proportions of nitrogen fractions in different fish, different bacterial flora and different initial conditions of environment. Possibly if absolute values for initial nitrogen fractions were established on a single product prior to change, and the rate of change followed, spoilage might be measured.

Almquist *(1)* expanded the nitrogen distribution by chemical tests into in vitro tests for indigestible nitrogen. This factor, brought into an equation of nitrogen fractions, has made the "protein quality index" a measure of digestibility, hydrolysis, autolysis and natural distribution. Indexes based on analytical tests

[a] Presented before the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 23, 1950.

for nitrogen fractions can be correlated with nutritive value only by biological verification and there is still no explanation of what happens when a change is noted.

Quantitative proportions of amino acids occupy the attention of many research and quality control workers today. While these determinations are imperfect, extensive checks between laboratories show an average coefficient of variation of 8.9 percent *(5, 11)*. Acceptable assay procedures which include strong acid and alkali hydrolysis show no significant lowering of amino acid content of fish products after canning. It seems likely that some measure of decomposition must take place before the percentage of amino acids reflects this change. In the case of tryptophan, which occurs in fish muscle slightly over 1 percent of the total protein, the coefficient of variation found in this work *(11)* was 17.5 percent which means that occasionally a single laboratory check on a processed product could be 0.65 percent or 1.35 percent and still not be significantly different from an initial value of 1.0 percent.

Block and Mitchell *(2)* have used amino acid composition to arrive at a "chemical score" in an attempt to correlate chemical composition with nutritive value. The score is based on the percentage of a limiting amino acid compared with the composition of whole egg protein taken as an optimum standard. An example of this measure is wheat protein which contains 63 percent less lysine than whole egg protein and this value subtracted from 100 gives a chemical score of 37. The work shows excellent agreement in most cases with protein efficiency ratios and biological values by the nitrogen balance method. Comparisons of chemical score with bioassays show a correlation coefficient of about 0.83. This means that seven out of ten changes in chemical score are due to variations in biological value but that three are not, due to error in measurements or the fact that no correlation exists.

The authors explain that, especially as influenced by heat, proteins may undergo changes, that impair their nutritive value without disturbing the amino acid composition as ordinarily determined. The same conclusions were reached by Clandinin *(3)* who found little difference in total amino acids after acid hydrolysis in five fish meals processed quite differently. On the basis of growth tests it would appear that these values do not predict nutritive value. Amino acids liberated by enzyme hydrolysis (hog intestinal mucosa for three days digestion) were depressed from 21.53 percent in the case of stack temperatures (flame drying method) of 185° F. (85° C.) to 2.99 percent at 220° F. (105° C.). Fish meals showing damage by growth tests and decreases in enzymatically available amino acids were supplemented with the suggested limiting amino acid with only partial success in restoring nutritive value.

Previous work of this same nature *(13)* brings out the fact that the total amino acid content of soy bean oil meals is not changed from the raw to the properly heated product and that in the case of overheating, only three essential amino acids, lysine, arginine and tryptophan, show a loss. In contrast, each essential amino acid showed increase by proper heat treatment and decrease by excessive heat when aliquots for the assay were taken from pancreatin digestions. Hydrolysis was carried to five days and the alpha amino nitrogen in the digested fraction (undigested proteins were cleared by precipitation with glacial acetic acid at the iso-electric point) was measured by the nitrous acid micro-method of Van Slyke.

Melnick and Oser *(10)* are also of the opinion that products may be improved or impaired in value although showing no change in amino acid composition. They propose a method of in vitro digestibility for following nutritive values in food processing. The method consists of digestion under toluene of a sample with suboptimum levels of pancreatin at the optimum pH with periodic formol titrations on aliquots. There is some question as to what is measured in this titration, but under the conditions of this work, starting at a pH of 7.0, adding formalin and carrying the titration to pH 9.0, it is a measure of free amino groups.

Proponents of the in vitro enzyme hydrolysis method as a measure of nutritive value contend that more important than amino acid composition or liberation of amino acids under optimum conditions of enzyme hydrolysis is a measure of the rate of liberation. Sensitive tests for protein denaturation can apparently be worked out by formol titrations showing percent digestion against time. In the work cited *(10)*, two samples of dried skimmilk, one freshly made and one after a year's storage, showed the same amino acid composition and coefficient of digestibility, yet an appreciable difference in biological value and rate of in vitro hydrolysis. For factory control of processing, this test, standardized to an enzyme and substrate level for each product, may have a distinct use. True, it measures only susceptibility of a material to pancreatin digestion and does not elucidate the problem of what happened if a change is noted. Accompanying amino acid assays could be run from aliquots and compared with initial content to study rates of amino acid liberation. For example, microbiologically active lysine was liberated from heat-treated casein at a much slower rate by this method of pancreatin digestion than from unheated casein. It was pointed out that the rate of release of an essential amino acid may be so slow as to limit its use in the gastrointestinal tract. If the in vitro rate of hydrolysis can predict this action it is more important than a measure of amino acids by acid hydrolysis or continued enzyme action, which may show optimum amounts available.

Another report along the same thought is by March, Stupich and Biely *(9)* suggesting a measure of nutritive value from the amino acid content based on total (acid) hydrolysis, corrected for indigestible protein (residue from pepsin digestion, factor "B" in Almquist's protein quality index) and non-protein fractions. Here, however, neither total nor available amino acid levels of different fish meals gave a satisfactory correlation with growth response in chicks. Good correlations were found between available tryptophan times digestible protein-nitrogen, and chick growth, and between available methionine plus cystine times digestible protein-nitrogen, and chick growth.

Freed, Brenner and Fevold *(6)*, using in vitro pancreatin hydrolysis of protein as a digestibility measure,

have eliminated the variability in expressing results by rate of digestion or titration values. They find a constant index regardless of sample weight or time of digestion in a formula "percent hydrolysis/gram$^{1/3}$ protein x days$^{1/3}$." They emphasize the fact that this digestion index is not necessarily analogous to biological evaluation.

We do not know from this technic whether different values mean a destruction of amino acids, decomposition of the product, changes in solubility or actual changes in susceptibility to enzyme action. A process may destroy an essential amino acid and at the same time permit a better rate of hydrolysis in the remaining protein.

Rizzo, Davis and Smith *(4, 14)*, have shown that lactalbumin goes through a deleterious change when autoclaved at 248° F. (120° C.). They found decreased digestibility (nitrogen excreted per gram food consumed), decreased formol titration values (nitrogen in supernatant fluid after in vitro digestion) and decreased biological values (change in weight per gram food eaten) and concluded that this change is due to a lowered susceptibility to enzymatic hydrolysis. Formol titration on pancreatin hydrolysates showed very nicely that this change is more rapid when the protein is autoclaved than when baked in a dry oven.

Enzyme activity has been suggested as a measure of general protein metabolism or possibly amino acid availability. Williams and Elvehjem *(16)* working with experimental animals and cognizant of the fact that gross body changes are not usually sensitive enough to reflect small differences in protein metabolism, have measured the relation of liver xanthine oxidase activity to dietary protein. Xanthine oxidase is a flavo-protein, the prosthetic group of which consists in part of riboflavin-adenine dinucleotide. Its decreased activity may indicate incomplete assimilation of amino acids or incomplete digestion of a protein. Using adult male rats of uniform strain and measuring enzyme activity in $\mu l\ 0_2$ per gram liver protein, a 14.6 percent casein ration showed less than normal activity. The addition of 0.25 percent methionine to the basal casein ration, an isonitrogenous level of acid-hydrolyzed casein adjusted for tryptophan, a purified amino acid mixture corresponding to the basic ration and 40 percent casein, produced normal enzyme activity. The conclusions were that xanthine oxidase activity was subnormal on a 14.6 percent casein diet because the animals were unable to utilize all the methionine and that this was due to the incomplete digestion of the protein.

Research in the writer's laboratory on the effects of processing on fish protein as measured by amino acid content, digestibility by formal titration, rate of liberation of essential amino acids by pancreatin and chick growth, will be presented in a later paper. Results so far indicate that where chick growth and feed efficiency comparisons are taken as accurate expressions of nutritive value, the essential amino acid composition expressed as a chemical score and re-expressed as a biological value by regression equations, accurately reflects changes in fish protein due to processing. It does not reflect the comparative value of casein or casein supplemented with cystine as related to fish protein. Neither rate of essential amino acid liberation in pancreatin digests nor digestibility by formal titration indicates essential amino acid deficiencies which were reflected in the chemical score and chick assay.

With pressure to develop new processes from the "customer acceptance" and the "economics of processing" standpoints, it seems essential in the interests of science that food technologists have methods of following the nutritive value as an aid to the utilization of a product. We know that there is some change in proteins when enzymes are inactivated, when bacteria lose their power to reproduce, in short, when preservation is effected. We want to produce processed foods of as high or higher nutritive value, if possible, than they had in the original condition. There is evidence that some means of preservation makes proteins more susceptible to enzyme action than they are in the native form. The volume of recent literature on nutritive values of foods as influenced by processing shows that the importance of this subject is recognized by food technologists.

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Improving Quality of Frozen Strawberries With Added Colloids

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Tennessee Supreme strawberries treated before freezing with from 0.1 percent to 0.4 percent of various colloids in water solution were definitely improved in quality.

INTRODUCTION

Frozen strawberries would be more pleasing to the average consumer if they were firmer and contained less free juice. Firmer berries would more nearly approach the character of the fresh berries. Excessive juiciness in a package tends to make the consumer feel that there are less berries, or that water has been added.

A previous investigation at the TVA Food Laboratory showed that calcium salts had no appreciable firming effect, but that certain pectinates and alginates did increase the drained weights of the defrosted products *(2)*.

In an earlier study, Baker *(1)* found that the use of cold 2 percent pectin of about 5 percent methoxyl content reduced the loss of juice from thawed strawberries by 33 percent. In no instance did small amounts of calcium chloride alone, or in sequence with low methoxyl pectin, equal in prefreezing value the use of pectin alone.

Since the previous work had shown promising results, more extensive tests using Tennessee Supreme strawberries were conducted during the 1949 strawberry season.

EXPERIMENTAL METHODS

The strawberries were prepared, using the various treatments, then packed in one-pound containers consisting of a waxed carton with liner and waxed overwrap. The dry sugar was added at the rate of one part for four parts of strawberries (4 + 1) for one-half of the samples and as a 60 percent sirup at the rate of one part per three parts of berries (3 + 1) for the other half. This was done with whole berries as well as using sliced berries. Each lot was treated with three colloidal products at levels of 0.1, 0.2, 0.3 and 0.4 percent by weight of the fruit. The three products used were: (1) a low methoxyl pectin; (2) a kelp extractive (propylene glycol alginate); (3) an Irish moss extractive.

In the dry dispersion method of adding colloids to the fruit, the calculated amount of colloid was thoroughly mixed with the dry sugar and this mixture added to the berries at the designated rate. The water dispersion method of colloid addition was accomplished by heating the water to approximately 71° C. (160° F.) to be used in preparing the 60 percent sucrose sirup, adding the dry colloid with vigorous stirring, and then mixing the quantity of sugar used in the sirup formula with the hydrated colloid.

Sufficient number of control samples were packed so as to have duplicate controls for each group of samples. A group consisted of one type of pack having 12 different colloid treatments, prepared by adding each of three different colloids at four concentrations.

The samples were frozen in a tunnel, using air at —46° C. (—50° F.) and 1,000 f.p.m. They were removed upon reaching —18 to —23° C. (0 to —10° F.) and placed in a —23° C. storage room.

After four months' storage the samples were weighed then removed to a 21° C. (70° F.) room for defrosting. When the centers of the packages reached 10-16° C. (50-60° F.), the fruit was drained for two minutes on an 8-mesh screen. The drained weight of the fruit and the volume, weight and percent of soluble solids of the drained juice were recorded. Organoleptic evaluations were made of the appearance, color, flavor and texture. The packages had been given code numbers so that their identity was unknown during examination and evaluation.

Since it was not possible to make all the tests on the 12 packages of a group in a very short time, the period of defrosting and final temperature before draining varied. To determine the effect of these temperature differences on percent drained weight, samples of "4 + 1" sliced strawberries with and without a colloid additive were thawed and drained weights taken at intervals of about 2.8° C. (5° F.) from 7.2° to 18.3° C. (Figure 13).* Within a 11° C. temperature range, there was a 9 percent difference in percent drained weight for the samples containing 0.4 percent low methoxyl pectin, and a 5 percent difference for the samples containing no pectin. This is approximately 0.45 and 0.25 percent change in percent drained weight for each degree of (F.) temperature.

To further examine the effects of the additives under extreme conditions of handling, drained weights were redetermined after the drained fruit from each type of pack was allowed to remain on a plate for two additional hours at room temperature.

RESULTS

The increases in percent drained weights in the sirup-packed berries, particularly those in the whole state (Figure 3), were greater than in the sugar packs. In

* The curve in Figure 1 was plotted by linear regression because the points were too scattered to attempt a visual approximation. All other curves were drawn as indicated by their visual pattern.

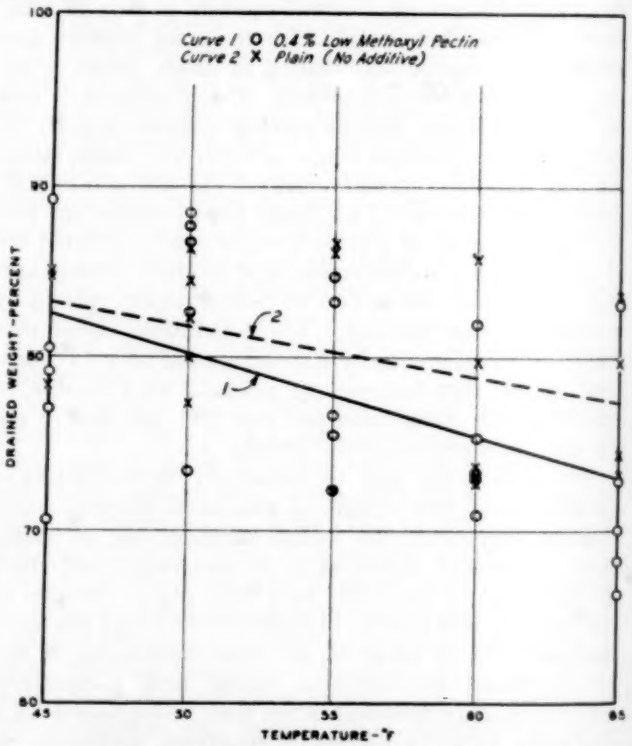

FIG. 1. Effect of temperature on percent drained weight of frozen sliced strawberries with and without low methoxyl pectin. (4 + 1 sugar).

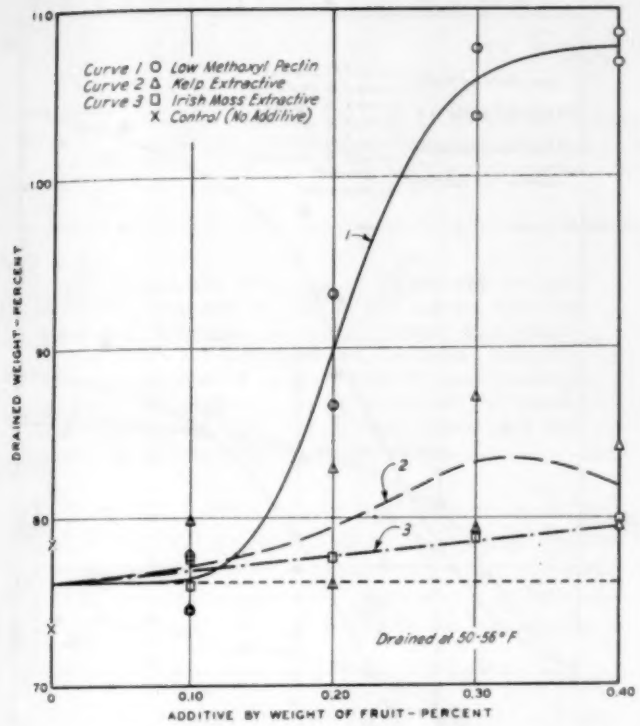
Curve 1 ○ Low Methoxyl Pectin
Curve 2 △ Kelp Extractive
Curve 3 □ Irish Moss Extractive
X Control (No Additive)
DRAINED WEIGHT - PERCENT
ADDITIVE BY WEIGHT OF FRUIT - PERCENT
Drained at 50-56° F

FIG. 2. Percent drained weight of frozen sliced strawberries with added pectinate and alginates. (3 + 1, 60 percent sirup).

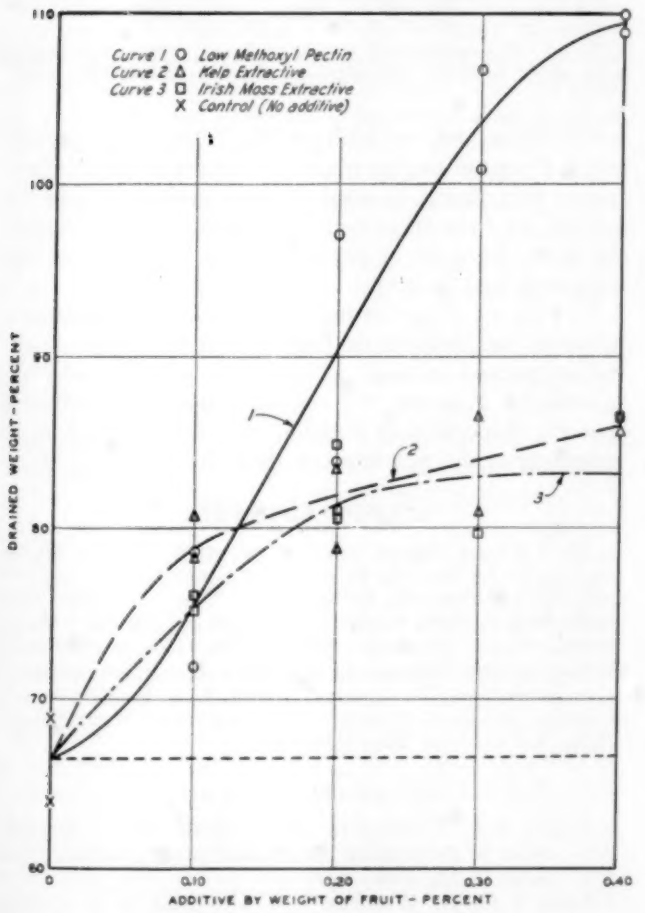
Curve 1 ○ Low Methoxyl Pectin
Curve 2 △ Kelp Extractive
Curve 3 □ Irish Moss Extractive
X Control (No additive)
DRAINED WEIGHT - PERCENT
ADDITIVE BY WEIGHT OF FRUIT - PERCENT

FIG. 3. Percent drained weight of frozen whole strawberries with added pectinate and alginates. (3 + 1, 60 percent sirup).

general, there was no consistent relationship between the percent drained weight of the samples with colloids added to dry sugar and to the amount of additive. Obviously, the colloids are much more effective when first dispersed in a liquid before being added to the fruit. This is in agreement with the manufacturer's recommendations.

For the "3 + 1" 60 percent sirup berries, the difference between the control and the fruit with colloids was not as noticeable in the sliced as in the whole berries (except for pectin). This was due to the fact that the control sliced sample had a percent drained weight 10 percent higher than the control whole samples (76 and 66 percent). The 0.1 percent and 0.2 percent samples of the three colloids in the whole "3 + 1" sirup packs had percent drained weight well above that of the control. The drainage temperature range observed does not account for an increase of 10 to 25 percent in percent drained weight. A level of 0.15 to 0.20 percent of low methoxyl pectin increased the drained weight of the sirup samples as much as the 0.3 and 0.4 percent levels of the other additives.

In the water dispersion method of adding the colloids to the fruit, all of the 0.3 and 0.4 percent low methoxyl pectin samples contained a gel at the time of draining. A few at the 0.2 percent level showed a tendency to gel. The other colloids did not gel at any of the levels used, but their 0.3 and 0.4 percent water dispersion mixtures were extremely viscous and difficult to handle in the packaging procedure. At the 0.1 percent level the three colloids produced approximately the same increase in percent drained weight.

Figures 4 and 5 (results of redraining) show that even under these conditions the sirup samples with added colloids maintained higher percent drained weights than the control and that each colloid remained in its relative position of effectiveness. However, the colloid-sugar packs did not maintain their relative positions after the redrain, and some samples had lower percent drained weights than the control.

Duplicate organoleptic evaluations of each group of samples were made by five examiners. The results showed that the colloids improved the defrosted fruit by producing a glossy appearance and a slightly firmer texture. In all of the sliced fruit and some of the whole, the control was described as being the dullest and softest among a group of unidentified samples. The flavor remained unchanged by the addition of the colloids.

The percent soluble solids of the drained juices were within the usual range for these fruit-sugar ratios, and were not significant in this work. The red color in the drained juices was measured by a photoelectric colorimeter with a filter at 425 millimicrons.[b] In most cases, the juice from samples containing added colloids had a greater concentration of red color than the juice from the control samples. The color values showed no significant differences between the type packs, the colloids and/or their varying levels of concentrations. As was expected, the percent drained juice for all type packs

[b] Analyses made by Dr. Glenn H. Joseph, Research Department, California Fruit Growers Exchange, Corona, California.

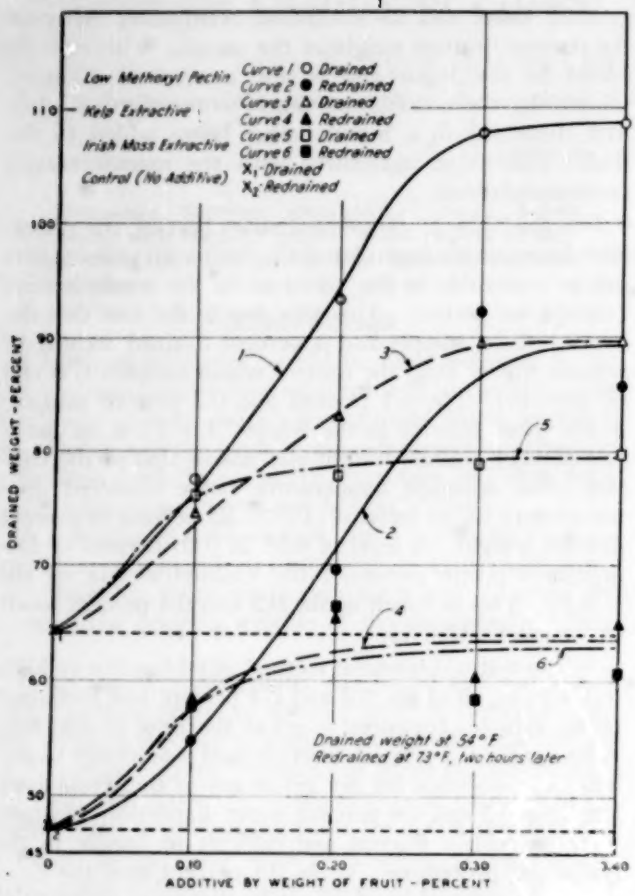

FIG. 4. Percent drained weight of frozen sliced strawberries with added pectinate and alginates. (3 + 1, 60 percent sirup).

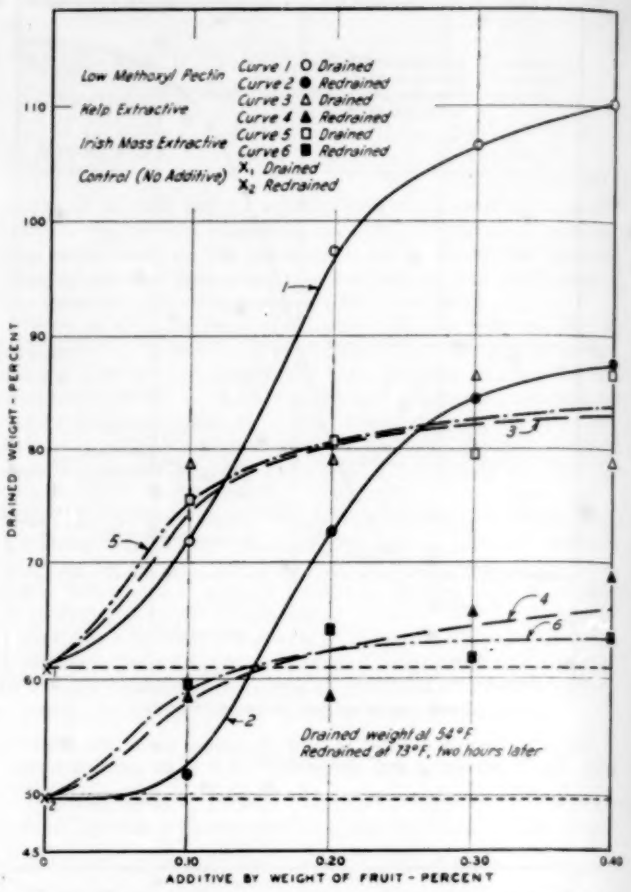

FIG. 5. Percent drained weight of frozen whole strawberries with added pectinate and alginate. (3 + 1, 60 percent sirup).

was inversely proportional to the percent drained weights. This same relationship was observed in a previous investigation.

SUMMARY AND CONCLUSIONS

1. Various concentrations of a low methoxyl pectin, a kelp extractive and an Irish moss extractive were used in the preparation of whole and sliced strawberries packed with sugar and sirup.

2. The samples packed with sirup containing the colloids mixed by water disperson showed marked increases in drained weights. The results obtained in the drained weights of the sugar-packed fruit with added colloids were inconsistent and erratic. Thus most effective use is obtained by dispersing them in water to be used for the preparation of sirup.

3. The results of the organoleptic examinations showed that the three colloids improved the appearance of the defrosted fruit by adding a gloss or sheen, and by producing better shape retention of the berries.

4. The pectinate product was the most effective from the standpoint of increasing drained weight to an optimum with the smallest quantity of colloid. A level of 0.15 percent to 0.20 percent of low methoxyl pectin increased the drained weight of the sirup samples as much as the 0.3 and 0.4 percent levels of the other additives. Because occasional gel formation appeared with 0.2 percent of low methoxyl pectin and because it is not known from this study the exact level at which gelation would occur, only an interpolation between 0.1 percent and 0.2 percent can be made for the maximum concentration that should be used to avoid gelling. At the 0.1 percent level the three colloids produced approximately the same increase in percent drained weights of the berries packed in sirup.

5. This investigation has shown that the appearance, firmness and percent drained weight of frozen strawberries packed in sirup is improved with the addition of colloids. However, the results of this study indicated that the dispersion of colloids in a dry sugar pack of strawberries did not improve their drained weights.

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A Comparison of Sensory Methods of Measuring Differences in Food Qualities[a]

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The comparative precisions of paired and triangle difference tests and of ranking and scoring tests for measuring differences in certain quality characteristics of foods are illustrated and discussed. Information is also presented on the ability of panel members to duplicate judgments and on preference for chocolate fudge made with and without vanilla and for apple juice of varying degrees of acidity.

At the recent conference on Methodology in Palatability Testing held in Washington, D. C., several taste tests were conducted to illustrate procedures in common usage and to compare the precision of results obtained by different sensory methods for measuring a food quality.

Paired and triangle difference methods, previously described and used by Bengtsson and Helm *(1)*, Boggs and Hanson *(2)*, and Peryam and Swartz *(3)*, were used to compare the flavor of chocolate fudge made with and without a standard amount of vanilla flavoring. Ranking and scoring methods described by Boggs and Hanson *(2)* were used to measure differences in acidity of apple juice containing varying amounts of added lemon juice. Samples for the comparative tests on any one food were treated the same or taken from the same lot, and were rated by 55 to 70 persons in attendance at the palatability conference.

For the paired difference test, two samples of chocolate fudge, one with and the other without vanilla, were coded and served on a paper plate. The tasters indicated on a score card which of the two samples had an added flavoring ingredient and also which one they preferred.

One-half hour later, three samples of the same fudge, two samples without vanilla and one with vanilla, were served in a similar manner for a triangle test. The judges were asked if there was a difference among the three samples and, if so, which two were identical. Again, they were asked to indicate their preference.

For the ranking and scoring tests, three samples of commercially bottled apple juice containing 0, 1, and 4 percent lemon juice were used. A 1-ounce (28 ml.) portion of each was served in a coded paper cup at room temperature. Each taster was asked to rank the three samples of juice in order of increasing acidity and to indicate which sample he preferred. Incidental to the ranking test, two questions were asked, "Is the amount served adequate for the test?," and "Is the number of samples too few, just right, or too many?" After each taster had completed the ranking test, he was asked to score a second set of three samples on a 10-point scale of acidity with 10 as the highest acidity and 1, the lowest. The apple juice without added lemon juice served both as a known reference standard and an unknown in the numerical scoring test. No standard was used in the ranking test.

DISCUSSION OF RESULTS

Paired and Triangle Difference Tests. In the paired difference test on chocolate fudge made with and without vanilla, the probability of selecting the sample with the added flavor by chance was one-half. Of the 70 persons who participated in the test, 35 could be expected to identify the sample correctly by chance. Actually, 50 (71.4 percent) identified the sample correctly, 15 incorrectly, and five found no difference. In the analysis of results, these five were divided between groups judging correctly and incorrectly, 2.5 being added to each. The departures from the expected values were highly significant as determined by the chi square test. The probability of the observed number of tasters selecting the correct samples by chance alone was considerably less than 1 in 100.

In the triangle difference test on the chocolate fudge, the probability of selecting the correct duplicate samples was one-third. In this instance, 48 (71.6 percent) of the 67 participants identified the duplicates correctly; 10 paired the samples incorrectly, 8 found no flavor differences among the samples and one found all three different. These nine judges were added to the remaining groups, one-third to the group who judged the duplicates correctly, and two-thirds to the group who paired samples incorrectly. The number of judges identifying groups correctly departed significantly from the expected number (22) who might have judged correctly through chance alone. The probability of obtaining these results through chance alone was considerably less than 1 in 100.

TABLE 1

Results of Identification of Vanilla Flavoring in Chocolate Fudge by Paired and Triangle Tests

Classification of Judges	Judges	
	Number	*Percent*
Discriminating judges		
Correct on paired and triangle tests	31	51.7
Non-discriminating judges		
Incorrect on paired and triangle tests	7	11.7
Correct on paired test, but incorrect on triangle	11	18.3
Correct on triangle, but incorrect on paired test	11	18.3
Total	60	100.0

Although the proportion of correct selections for all participants in either test was 71 percent, only 52 percent of the 60 judges who participated in both tests made correct selections on both the paired and triangle difference tests on chocolate fudge as shown in Table 1.

[a] Presented at the Tenth Annual Meeting of the I. F. T., Chicago, Illinois, May 24, 1950.

About 12 percent were incorrect on both tests. Eighteen percent of the judges were inconsistent in that they selected the samples correctly on the paired test but incorrectly on the triangle test. The same proportion made the correct selection on the triple comparison test but failed in the paired test. With *a priori* odds 2 to 1 in the triple comparison test, it would be anticipated that a correct selection in the triple comparison would be more difficult than in the paired test in which completely indiscriminating judges have a 50-50 chance of being successful. The triangle test would, therefore, be expected to eliminate a greater proportion of marginal individuals, i.e., those who pass one test and fail the other, than actually occurred. The even split of inconsistent choices on both tests as well as the equality of successful choices on individual tests (71 percent) indicates that for these persons, materials and conditions of judging, neither the paired nor triangle test was more precise than the other in a practical sense. Either could be used equally well in selecting panel members to judge vanilla flavoring in chocolate fudge. With better control of judging environment and sampling variation of materials than was attainable under the circumstances of these experiments, more of the marginal individuals might have failed the triangle test.

Table 2 shows the preference choices of judges in the chocolate fudge test. Of the 70 persons who participated in the paired comparison test, about 62 percent preferred the sample with vanilla added; 31 percent preferred the sample without vanilla and 7 percent expressed no preference.

TABLE 2

Preferences of Judges for Chocolate Fudge With or Without Vanilla Flavoring as Indicated by Paired and Triangle Tests

Preference	Paired Method		Triangle Method		Paired and Triangle Methods	
	Judges		Judges		Judges	
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Discriminating judges [b]						
Vanilla added	43	61.5	31	46.3	21	35.0
No vanilla added	22	31.4	16	23.8	6	10.0
No preference	5	7.1	1	1.5	2	3.3
Non-discriminating judges [c]	....	....	19	28.4	31	51.7
Total	70	100.0	67	100.0	60	100.0

[b] Judges who correctly identified samples and duplicated preference.
[c] Inconsistent in identification and/or preference.

In the triangle test, 46 percent of the participants identified the samples correctly and indicated a preference for the chocolate fudge with vanilla. Twenty-four percent preferred the fudge without vanilla. Only one judge expressed no preference. Twenty-eight percent failed to identify the samples correctly.

The reliability of the results in the triangle test is enhanced by the replication feature in that each judge had an opportunity to express a preference for two samples of material from the same source. Although the preference ratio is 2 to 1 for vanilla, the judges expressing no preference and those judging inconsistently probably are as important as the discriminating judges in an appraisal of food products from the standpoint of consumer preference.

Only 35 percent of the judges who participated in both the paired and triangle tests consistently preferred chocolate fudge with vanilla added. Ten percent consistently preferred chocolate fudge without vanilla and three percent expressed no preference. Fifty-two percent either could not duplicate their preference or correctly identify the samples.

Ranking and Scoring Tests. In the ranking of the three samples of apple juice containing 0, 1 and 4 percent lemon juice, 38 (69 percent) of the 55 judges who participated, ranked the samples correctly in ascending order of acidity. Three ranked samples in descending order. Three judges placed the standard correctly but reversed the other samples. The commonest mistake made was confusing the standard with the sample which contained 1 percent lemon juice, but placing the sample of highest acidity in its proper order. Testing results of ranking by analysis of variance showed that judges found the samples different at a high level of significance.

Fifty-six persons participated in the numerical scoring of three samples of apple juice. Thirty-five (62.5 percent) scored in the correct sequence of increasing acidity. The remainder assigned scores in wrong sequence or gave identical scores to the samples. As in the ranking test, the commonest mistake was confusing the unacidified juice with the sample which had one percent added lemon juice.

In spite of mistakes in scoring, the difference between the mean acidity scores for apple juice were in line with the differences between levels of acidity. The mean acidity score for apple juice without lemon juice was 3.89, for apple juice with 1 percent lemon juice, 4.96, and for apple juice with 4 percent lemon juice, 8.48.

Analysis of variance applied to the numerical scores showed that the panel found highly significant differences (beyond the 1 percent level) in acidity of the samples of apple juice. In the absence of replication, neither the judges nor their interaction with samples could be tested directly. However, a relatively high interaction mean square of judges and samples reflects cases of inverted order of scoring which occurred in the data.

Correct and incorrect scoring were compared for both methods of judging. When data were scored numerically, the proportion of correct answers was 62.5 percent. When the samples were ranked, 69 percent of the judges indicated the correct sequence. However, the difference between the two sets of figures was not significant at the 5 percent level as determined by the chi square test.

Fifty-four judges scored and ranked the apple juice samples as shown in Table 3. Fifty percent judged correctly in both tests. Nine (17 percent) judged incorrectly both times and 18 (33 percent) missed on one or the other of the tests. Eleven assigned a score other than 10 to the samples they chose as having the highest acidity, perhaps due to misunderstanding the instructions. This did not constitute a failure of the test, however, since the above comparison is based on whether the samples were ranked and scored in the correct order

TABLE 3

Results of Ranking and Scoring Tests on Samples of Apple Juice at Three Levels of Acidity

Classification of Judges	Judges	
	Number	*Percent*
Discriminating judges		
Correct on ranking and scoring	27	50.0
Non-discriminating judges		
Incorrect on ranking and scoring	9	16.7
Correct on scoring, incorrect on ranking	8	14.8
Correct on ranking, incorrect on scoring	10	18.5
Total	54	100.0

of increasing acidity. Six cases of inverted order were noted in the two tests, but it is unlikely that these were caused by uncertainty as to whether 1 or 3 was the rank for highest acidity for no judge made this error twice.

It is difficult to measure the relative precision of the results of ranking and scoring in the apple juice tests since no adequate measure of variation of the respective experimental errors is available. The comparison of the consistency of judging in Table 3 indicates substantially equal success in identification on both tests. If one test were much better than the other, it would be expected that a considerably greater percentage of correct answers would have been made on one test than on the other by persons who failed to be consistent in judging both by ranking and scoring.

The preferences in the ranking test were more easily tallied than in the numerical scoring test because answers were more decisive in the former. Twenty-two (40 percent) of the judges in the ranking test preferred the unacidified juice; 24 (44 percent) selected the slightly acid drink; 8, the highly acid drink and only one preferred both acid drinks over the unacidified juice. Each of the 55 judges expressed an opinion.

In the numerical scoring test, 13 of the 56 who participated in the scoring failed to answer the question as to preference of drink; one had no preference; one preferred "the standard," apparently unaware that it was identical with the unacidified sample. The remaining 41 judges selected as follows: 15 (37 percent) preferred the unacidified juice; 15 the slightly acid; 2 chose either the unacidified or slightly acid. Eight (20 percent) expressed a preference for the most acid and one preferred either the slightly acid or most acid.

The preferences of 40 judges for whom results were available in both ranking and scoring tests were compared. As shown in Table 4, 17 expressed a preference for the same sample each time, whether the sample was unacidified or one of the acidified juices. Twenty-three

TABLE 4

Preference of Judges for Different Degrees of Acidity in Apple Juice

Preference	Judges	
	Number	*Percent*
Discriminating judges [a]		
No added lemon juice	8	20.0
1 percent added lemon juice	6	15.0
4 percent added lemon juice	3	7.5
Non-discriminating judges [b]	23	57.5
Total	40	100.0

[a] Judges who duplicated preference on two tests.
[b] Inconsistent in preference.

were not consistent in their choice. The judges apparently experienced difficulty in differentiating between the unacidified and slightly acid juice since 16 selected the unacidified in one test and the slightly acid juice in the other.

With respect to questions asked regarding number and size of samples, the majority of the panel members (80 percent) responded that the number of samples (three) in the ranking test was "just right;" 11 percent said three samples were "too few," 9 percent thought three were "too many." Ninety-one percent of the panel replied that the amount served in each sample (28 ml.) was adequate; only 9 percent thought it was not. A few of the panel members remarked that they did not like the paper cups. However, for such a large number of samples (about 400), paper cups were far more convenient to use than other types of containers.

SUMMARY

Taste tests were conducted to compare the results obtained by different sensory methods of measuring certain food qualities. Paired and triangle methods were used to measure differences in flavor of chocolate fudge made with and without vanilla flavoring. Ranking and scoring methods were used to measure differences in acidity of apple juice containing varying amounts of added lemon juice. Samples for the comparative tests were rated by 55 to 70 persons.

In the paired and triangle difference tests on chocolate fudge, 71 percent of the participants identified the samples correctly in a single test, but only 52 percent judged correctly on both tests. When judgments were matched, as many judges gave incorrect answers on the triangle test as on the paired test. Therefore, one test was not more precise, in a practical sense, than the other as a basis for selecting reliable panel members. However, the triangle test inspires more confidence in the results because of the opportunity to eliminate judges who cannot identify duplicate samples.

Ranking and scoring procedures functioned equally well in detecting differences in acid concentration of apple juice. The scoring method, however, indicated degrees of difference that were not shown by ranking. Only 50 percent of the judges who both scored and ranked apple juice samples made identical correct selections.

Preference tests were of interest in showing that only 48 percent of the judges duplicated their preference for correctly identified samples of chocolate fudge and only 42 percent indicated preference for apple juice of the same degree of acidity on two different tests.

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Notes and Letters

The Effect of Subtilin and Heat in Preventing Spoilage of Comminuted Beef[a,b,c]

Recently Andersen and Michener *(1)* reported that the complementary action of subtilin and mild heat greatly reduced heat requirements for sterilization and gave promise of higher food quality in canned vegetables. Shortly after their paper was published, work was initiated at the Iowa State College on a series of studies using comminuted beef to determine whether addition of this antibiotic could be used to minimize the time required for heat processing of meats.

By use of a tube technique similar to that employed in thermal death time studies, it was possible to obtain information in a comparatively short time and with only a small amount of meat.

The reduction in thermal lag accomplished through use of 9 mm. pyrex tubes was considered advantageous for the application of heat. The beef samples were comminuted to provide maximum contact with heat and subtilin and to minimize variations due to sampling.

Canner's grade beef was trimmed and comminuted in a Hobart Silent Food Cutter. Aliquots of comminuted beef were inoculated with each of two spore concentrations (100 and 10,000 spores of N.C.A. Putrefactive Anaerobe No. 3679 per g.), four levels of subtilin (0, 10, 100 and 1,000 p.p.m.), and processed at temperatures of 212 and 240° F. (100 and 115.6° C.) using process times of 1, 5 and 10 minutes. Each of the 48 combinations of the 4 variables ($4 \times 2 \times 2 \times 3 = 48$) was replicated four times. Thermal death time tubes, 9 x 100 mm., were stuffed with comminuted beef by means of a spring-primed grease gun before vacuum sealing. Tubes were preheated at 176° F. (80° C.) for 5 minutes, processed in thermoregulated oil baths, and immediately cooled in ice water. The processed tubes were incubated at 98.6° F. (37° C.) and inspected periodically.

Spoilage was determined by changes in appearance, color, odor and texture. Generally, there was an initial reddish discoloration followed by gas production and darkening. The most useful criterion of beef spoilage was the degree of darkening. Tubes of meat were considered spoiled when the degree of darkening exceeded that of normal tubes. The results obtained are shown in Table 1.

TABLE 1

Percent Spoilage of Processed Beef Samples After 1½ Months of Incubation at 98.6° F. (37° C.)

Subtilin used, p.p.m.	Processed at					
	212° F. (100° C.)			240° F. (115.6° C.)		
	Process time, min.			Process time, min.		
	1	5	10	1	5	10
	Spore concentration: 10^3/g.					
0 [d]	100	100	100	100	100	12
10	100	100	100	100	70	20
100	87	80	53	87	6	0
1000	27	20	0	29	6	0
	Spore concentration: 10^4/g.					
0	100	100	100	100	100	100
10	100	100	100	100	100	81
100	100	100	100	100	83	6
1000	100	100	94	100	6	0

[d] All control samples except those processed for 10 minutes at 240° F. (115.6° C.) were spoiled within six days.

The majority of processed beef samples exhibited spoilage within 1½ months. Subtilin at high levels delayed spoilage but a constant increase in percent of spoilage with time of incubation suggests a loss in the stability of subtilin during storage or that subtilin activity might be involved in hindrance of spore germination rather than in spore destruction. The latter premise is in agreement with a recent publication of the National Canners Association *(2)*.

Further studies on this subject are now in progress and additional details will be published in a future report. However, the data already on hand indicate that a relatively high level of subtilin of the potency used[e] may be required to prevent spoilage of comminuted beef. Effective prediction of the effect of subtilin and heat on beef processed in cans could only be made upon completion of additional studies.

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2. National Canners Association. Information Letter No. 1287, p. 184 (1950).

[a] Journal Paper No. J-1854 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project Nos. 1017 and 1123.

[b] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned Number 321 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

[c] The physiological effects of subtilin and other antibiotics, if used in foods, has not been clearly established. Practical use of these agents in foods is not permitted at the present time.

[e] The potency of the subtilin used was 19,000 units per mg. The authors are indebted to Merck & Company, Rahway, N. J., for supplying and assaying the subtilin used, and to Dr. Hans Lineweaver, Western Regional Research Laboratory, Albany, Calif., for assaying the subtilin for potency.

Industrial and Legal Viewpoints

The Food Technologist in the Meat Canning Industry*

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To outline the role of the food technologist in the meat canning industry I think it would be in order to review briefly what he has accomplished in the past and consider some of the problems which he now faces and which he expects to solve in the future.

The first purpose of food canning is preservation of products in an edible state. From the time of Nicholas Appert the food technologist has devoted much effort to this problem and he has been very successful in solving it.

One of the factors responsible for a large reduction in the occurrence of spoiled canned meats has been a better understanding of the principles of sterilization. Processing times and temperatures have been established based upon carefully determined heat penetration data and a knowledge of the thermal death times of the organisms to be destroyed. This has eliminated much of the guess work in processing schedules. At the same time such equipment as automatic controls, timers, and recorders, has become standard for maintaining predetermined processing schedules.

Improvements in sealing compounds and better closing equipment have reduced the percentage of leakers to a small fraction of one percent.

Increasing knowledge of thermophilic spoilage has called attention to the importance of proper chilling of certain products, with reduction in spoilage from this source.

Mechanical vacuum systems and special lacquers have reduced the amount of discoloration due to incompletely removed air and to the action of products on the tinplate.

Everyone will admit that the food technologist has accomplished his primary purpose, that of preserving products in an edible condition, in a highly successful manner.

In recent years, in cooperation with his fellow worker the nutritionist, he has gone somewhat further and has concerned himself with the question, "Are all the original nutritive values of the product retained?" He has obtained partial answers to this question and additional information is appearing regularly.

The region where art and science meet offers the canned meat technologist his greatest challenge. In the years ahead he will be increasingly concerned with factors which affect the sales appeal of his product, with color, texture, and especially with flavor.

The canned meat industry is passing through a period of transition similar to the one the smoked meat industry passed through a generation or two ago. The first purpose of curing and smoking meats was preservation. Before the days of adequate refrigeration, the only way to preserve meat over a considerable period of time was to cure or salt it heavily and smoke it. The high salt content, long drying period, and preservative action of the smoke, produced products which kept reasonably well but which were salty, tough, and often of poor flavor. With the use of improved methods of preservation which followed the development of mechanical refrigeration, the original purpose of curing and smoking meats became unimportant. However, the salty, smoked flavor, was liked by many people and the demand for these products continued after satisfactory refrigeration became commonplace. The demand, however, became more critical. Excessive salt was not tolerated; cured meats became milder and milder until today they are again very perishable. Instead of preservation, the emphasis has been placed upon appetite appeal.

Canned meats are going through much the same evolution. Twenty-five years ago the quality of canned meats left much to be desired. The favored formulas for potted meat and vienna sausage, two of the larger selling canned meat items, were those using the cheapest ingredients. If the product did not blow up in the can, not much fault was found with it. In recent years this situation has been changing rapidly. Instead of the cheapest packinghouse by-products going into cans, some of the most expensive cuts such as hams, account for a large percentage of today's canned meat business. Last year 123,000,000 pounds of canned ham were produced in this country. This is over 10 percent of the total production of canned meats. Even the lower cost items such as vienna sausage and potted meat have been greatly improved in quality.

This change to better quality and better canned meat products was greatly accelerated during the last war. The armed forces not only demanded the highest quality of canned meats but insisted upon varieties which had never been packed before. The general public has taken up the demand and we find an increasing variety of canned meats in our retail stores. The demand for a better appearance and a better flavor continues.

This presents a challenge to food technologists. The high temperature necessary to sterilize canned meats presents some difficulties with respect to appearance and flavor. When we consider that a well done roast reaches an internal temperature of about 180° F. (82° C.) and that the center temperature necessary for sterilization of many meat products is about 50° F. higher than this, we begin to appreciate the gravity of the problem.

The moisture content of lean meat is about 75 percent. When meat is cooked the muscle fibers contract

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and a substantial part of the moisture is released. When fatter meat is used, the fat which melts in the neighborhood of 100° F. (38° C.) cooks out in the same manner. To maintain a satisfactory appearance in a fully sterilized meat product under these conditions is indeed a problem. During the last 10 or 15 years a considerable degree of success has rewarded the efforts of meat canning technologists in the sterilization of comminuted meats in small containers such as the 12-oz. luncheon pack. The problem still exists with regard to larger units such as a sterilized canned ham. Perhaps a solution of the problems of preservation by means other than heat sterilization will some day provide the answer. The use of antibiotics and of high energy cathode rays for sterilization offer exciting possibilities although it is emphasized that long periods of testing will be required before commercial utilization can even be considered.

More subtle in nature are the changes produced in flavor by the sterilization process. Many spice flavors are reduced in intensity by the heat treatment. This calls for a larger percentage of spice and at times different proportions of spices in the canned product. Some spice flavors are so adversely affected by processing that they become objectionable. This is particularly true with cured meats; the nitrite they contain is chemically reactive at high temperatures and will sometimes produce disagreeable flavors. The flavor of a smoked meat product is sometimes adversely altered by processing; a pronounced smoky flavor can become a bitter flavor.

Flavor changes also take place in canned meats when stored for long periods. Some of these changes are caused by interaction of the tin plate with the product. The use of enamel lined cans has sometimes helped. Other changes which occur in the flavor of products can be determined only by storage tests and periodic sampling. The solution of these problems is a large field of work for food technologists in the canned meat industry. We might ask: "Is it worth the effort? Why do people buy canned meats when fresh meats are available?"

The first purpose of canning meats, namely preservation, is still responsible for the sale of millions of pounds. The armed forces, for instance, are large purchasers of canned meats primarily because of this factor. Refrigeration is not always available to all segments of our civilian population. Vacationists and out-of-door enthusiasts are substantial users of canned meats. The stay-at-home will often stock canned meats for use in emergencies. However, we in the canned meat industry like to look upon canned meats not as emergency products but as staple items in the everyday diet. Convenience is a compelling motive in the purchase of many canned meats. Contrast, for instance, the time required to purchase the many ingredients which go into a meat stew; the cleaning of the vegetables, the proper seasoning and the long cooking period necessary to obtain a satisfactory flavor, with the ease of opening and heating a can of the various meat stews now readily available.

Uniformity of quality can be controlled far more closely in canned meats than in fresh meats which are sometimes subjected to temperatures and holding times not under control by the producer.

The total canned meat business of all establishments operating under Bureau of Animal Industry inspection in 1949 was 1,186,000,000 pounds. Since practically all meat canners do an interstate business, this figure is very close to the total canned meat production. Of this amount 375,000,000 pounds was in institutional sizes; that is, in units of 3 lbs. or more. By far the largest percentage of the institutional pack was luncheon meat and canned hams. These two items accounted for 317,000,000 pounds or almost 85 percent of the total. These products are usually pasteurized only and must be kept under refrigeration. The fact that 85 percent of the production of canned meats produced in large size cans must be kept under refrigeration emphasizes the difficulty of producing satisfactory sterile products in large units.

The balance of the canned meat production in 1949, about 711,000,000 pounds, was in consumer sizes weighing 3 pounds or less. All of this represents fully processed or shelf items. In the Bureau of Animal Industry report on the production of canned meats in 1949, luncheon meat in the familiar 12 oz. size heads the list with a production of 102,000,000 pounds. Following in order are chili, corned beef hash, vienna sausage, meat stew of all types, potted meats, spaghetti and meat, tamales, hams (whole and fractional), deviled ham, sliced dried beef, liver products, tongue and vinegar pickled products.

It is interesting to note the position of 12 oz. luncheon meats as the leading individual item. Fifteen years ago a sterile canned luncheon meat was practically unknown. Attempts to produce such a product with the knowledge then available resulted in a dry, soft textured meat, with three to four ounces of cooked-out fat and juice in a 12 oz. can. By developing the necessary "know how," the food technologist has so improved this product that only a fraction of an ounce of cooked-out fat and juice is found, and the meat has a firm, chewy texture. This improvement in quality has jumped the 12 oz. luncheon meat to a commanding lead over such long established items as chili, hash, vienna sausage and potted meat.

In addition to the products mentioned above, 209,000,000 pounds of other miscellaneous canned products were produced in 1949. To mention a few of them: Wieners, pork sausage, beef and gravy, pork and gravy, chop suey, hamburger, chicken, ham salad, barbecued beef and pork, chicken a la king, ham a la king, pureed meats for babies, beef and noodles, ham and lima beans, and many others. Some of these are newcomers into the family of canned meats and it is quite possible that ten years from now, one of them, or one still unknown today, will meet with consumer acceptance to such a degree that it will jump to the head of the list now held by luncheon meat. The possibilities offer an exciting challenge to food technologists in the canned meat industry.